

Bush declares war on drugs

This week's strategy for saving the United States from a new wave of drug dependency may create a sense that something is being done, but the real war will have to be won by different means.

PRESIDENT George Bush is nothing if not transparent. His declaration of war on narcotic drugs this week is what his electors have been looking for. There are three categories of people in the United States pleading for "tougher" action on drugs: middle-class parents fearing their children will be hooked, poor people in the city ghettos whose families and communities are being destroyed by the drug traffic and the generality of those who believe that the law is the law, and must be strictly enforced. This week's package will bring temporary comfort to them all, but will solve few of the problems that matter.

The one obvious exception is the administration's swift decision to provide the government of Colombia with military assistance to resist the outrageous anarchic ambitions of those called the drug barons, who control the cocaine trade. It will strengthen the reputation of the United States throughout Latin America if it is seen to be ready to help governments so seized of the danger that their constitutional foundations will be undermined that they appeal for assistance, as Colombia did two weeks ago. (Nicaragua is, or was, another matter.)

The social damage done by drugs in the United States has two aspects: that caused by narcotic addiction (or even by routine use) and that caused by the traffic. Bush plans to deal with both punitively. There will be tougher penalties for those convicted of selling or handling narcotics (and more people to help find them), and tougher penalties for those found using illegal narcotics. Coca-growing countries in Latin America will come under greater pressure from the United States to persuade their farmers to grow other crops, just as farmers in Turkey and Thailand a decade ago were being urged not to grow opium poppies.

Prohibition

The obvious weakness in this strategy is that attempts to prevent narcotics reaching the United States cannot reduce the supply to zero, just as the Strategic Defense Initiative cannot realistically be designed to destroy every single hostile missile. Will the success of the supply-side proposals be measured by the increase of street prices, which presumably will mean that fewer addicts will have to steal more cash to get their fixes? The attempt to frighten off drug users by devices such as the withdrawal of driving licences (whose constitutional legality is dubious) may work with some, but not in the city ghettos.

No wonder that comparisons with the fruitless war against alcohol in the 1920s, under the label of Prohibition, have been commonplace in the past few weeks.

But what else can be done? The administration's dilemma is admittedly forbidding. Taking a leaf out of the Prohibition book and decriminalizing narcotics would at this stage do much more harm than good, yet in the long run, that is what even United States government will have to come to. Given the sheer diversity of narcotics and the ease with which new materials can be synthesized if products based on agriculture dry up, punitive regulation cannot ultimately succeed. One obvious benefit of eventual legalization is that, by taxation, the financial beneficiaries are not distant drug barons, but governments which, if sympathetic, can use the funds they collect to help drug users and especially addicts overcome their difficulties. By the same stroke of the pen, organized crime is weakened. And would not a society in which narcotics were accessible that did not destroy itself by addiction be a stronger society, and perhaps even "kinder and gentler" as well?

Desolation

The most disappointing feature of the Bush proposals is that they do very little to point towards that goal. Drug dependency is probably always a consequence of personal desolation, the causes of which are plain among the poverty-stricken of the city ghettos. (The middle classes have subtle ways of concealing discontent.) Education and jobs, or some hope thereof, would help in the short run and are a precondition of decriminalizing the narcotics business. Sadly, in the United States, that option is denied even at the beginning of what was meant to be a presidency given over to educational improvement by, among other things, the federal budget deficit, which is but another way in which the United States collectively "takes the waiting out of wanting" — a fix of a different kind.

That is why it must be hoped that governments other than that of the United States, in Western Europe for example, will resist the pressure to which they will now be subjected to fall in with the Bush strategy as if it were a kind of extension of the doings of the North Atlantic Treaty Organization into the social field. There is nothing wrong with the hope that organized crime, national as well as international, can be beaten in its own right, while



smuggled drugs should be seized when found so long as they remain illegal. But there are many places in which the use of narcotics is still so modest that its further growth would most effectively be restrained by some system in which registered users can be licitly supplied — and given such help as there is. The British system for dealing with morphine and heroin addicts in such a way was too hastily abandoned 20 years ago when a single physician was found to be rashly over-subscribing. Paradoxically, the place to restart is with the hardest drugs, and where addiction is least prevalent. Even the United States would benefit if some government were to give that strategy a try. □

The Matthew principle

The managers of Britain's universities need devices for offsetting the attractions of successful institutions.

AT the beginning of another academic year, it is natural enough that the attention researchers pay to the last research meetings of the season should be inclined to wander, but British academics are more likely to let their thoughts wander than those from elsewhere. They have a great deal on their minds, not least the outcome of the long and elaborate survey of research performance at British universities (costing about £4 million) begun by the University Grants Committee and published two weeks ago by its successor, the Universities (no apostrophe) Funding Council (UFC). The objective, as the covering letter to university vice-chancellors explains, is to "inform" them about UFC's decisions about public subvention of particular universities in next year's academic year and in the succeeding four-year period. People will be anxious to know as soon as possible not only how their own departments showed in the assessments, but also how rival departments elsewhere are rated to have performed. Their future as researchers may depend on what the figures show.

But there are few surprises. The assessment is concerned exclusively with original research, not with teaching or with unoriginal contract work for outsiders. Assessments have been carried out by panels of active academics together with a sprinkling of retired people on the basis of information supplied by university departments through their universities. UFC says that more attention has been paid to research output — publications — than in previous exercises on a smaller scale by UFC's predecessor, but the details of how that has been done will not be arguable until the new academic year is well under way (but then there are certain to be ructions). And the result? Britain has two institutions with all-round excellence in research — Cambridge and Oxford. Some others (such as Imperial and University colleges, both part of the University of London) run them close. Then there is a solid core of civic universities (typified by Manchester) whose research performance is creditable on UFC's five-point

scale. And then there are stragglers in plenty.

Academics everywhere will know the explanation — it is nothing but the working of the Matthew principle of "To him who hath shall be given...". Potentially able people naturally seek posts at universities with a reputation in research and, equally, are sought to fill them. But a university already strong in research is usually better able to provide its members with opportunities for research while its reputation inevitably rubs off on them when, for example, they apply for research grants. What UFC now plans is that the Matthew principle shall be reinforced by a system under which the universities that are already strong will be further strengthened with resources at the expense of weaker places elsewhere. There is nothing intrinsically evil in that decision; it is only equitable that strong research universities should be given the resources required to match their unavoidable overheads. But the result must be to reinforce their primacy further.

Yet UFC has (or should have) a wider interest to ensure that universities further down the pecking order have a continuing opportunity to improve their research performance and thereby, for as long as British universities must depend on public funds, their income. How will that be accomplished? Much will depend on the way in which future public subvention of universities is "informed" by this year's assessments. There will be a temptation for UFC to assert that the research output of some university departments is so meagre that they should give up trying, perhaps concentrating on teaching instead.

That would carry the Matthew principle too far and confirm today's high-fliers in their present positions for the rest of time. At the very least, UFC needs to keep back some of its funds in each of the next several years so as to back research initiatives at the now less well favoured universities. But it must also make plain its determination to help the second and even third-rate institutions become first-rate if they can. One practical objective must be to keep the system competitive. Another, more subtle, stems from the reason why many academics follow what is (in Britain) a badly paid and generally thankless profession: there is always the opportunity for research. If that should ever disappear, many of the universities destined to become more concerned with teaching than research will quickly find themselves bereft of teachers.

The chances are that neither the British government nor the committees such as UFC which it appoints to do its work are conscious of that danger. Certainly the government, believing as it does that academics are layabouts and persuaded by its disdainful convictions that everybody else would wish to be a layabout if he had the chance, behaves as if the supply of academics is literally endless. The logic is faultless, but the premises are wrong. Even academics will not endlessly put up with the self-abnegation required of British academics in the past decade. Push them a little harder and they will move into the City of London — or even politics. How will UFC plan for that. □

Space station the victim of US budget difficulties

- International collaboration in the balance
- Reduced capabilities for early missions

Washington

REPRESENTATIVES from NASA (National Aeronautics and Space Administration), ESA (European Space Agency) and Japan's Science and Technology Agency are meeting this week in Paris to discuss plans to scale back the space station Freedom in anticipation of cuts in NASA's budget. US proposals to "rephrase" the space-station programme have angered its international partners, who say that if the United States breaks the terms of the intergovernmental memoranda signed last year it will jeopardize future international cooperation in space.

Under discussion in Paris will be plans completed last month by researchers at NASA's Langley Research Station. Ray Hook, director for space at Langley, says the group tried to find a way to cut development costs by 20 per cent and to produce a programme that would "eventually achieve the capacity that the current configuration has but at some later date". The plans are made necessary by budget restrictions. The House of Representatives has already cut \$400 million from its 1990 request of about \$2,000 million for the space station and the Senate may take similar action in the next few weeks.

Bill Oran, of the House of Representatives subcommittee on space science and applications, says that, further into the future, "the budgetary pressures are not going to go away". The international partners are up in arms over the proposed changes. Heinz Seipel, science counsellor at the West German Embassy in Washington said they would create "tremendous uncertainty" in the dates for attachment of the modules and in the dates when full power would be achieved. "The international partners have to make investments in billions . . . and rely on promises. That just one year after the agreements have been signed there are cutbacks does not increase the confidence of the partners in the stability of the system", he said.

Takehiko Kato, US liaison officer for the Japanese National Space Development Agency says the changes would cause "big problems" for Japan's module and that the number of experiments would have to be reduced. If the United States cannot honour the agreement signed last year, "nobody's going to work with the US any more because they're so unreliable", he said. Japan has complained formally to the United States and is awaiting a response at the meeting this week.

The new plans include drastic reduc-

tions in power, data-management capabilities and the number of personnel on board during the initial stages of the programme. But Hook says the programme could be restored to full capacity by 2000, two years behind schedule.

Other possible changes include: reducing communication and tracking capacity; minimizing or abandoning plans to allow the crew to work outside the space station; reducing navigation and control capability; using different life-support systems.

Instead of eight crew members there would only be four and power would be reduced from 75 kW to 38 kW. Of this only 18 kW would be available for users, instead of 45 kW. A spokesman for NASA said that if research projects are re-scheduled to make maximum use of the available power, then research will not have to be cut back. Hook said that although some experiments would be deferred, some of the instruments would not have been ready in time to meet the original schedule.

Inadequate power is one of the problems identified by a committee convened by the National Research Council at the request of NASA to study the engineering and design of the space station. In its report, published last month, the committee says the estimated power requirements were too low for the 'housekeeping' systems and the research projects.

Under the plan put forward by Langley, the date for first launch remains as scheduled, in the first quarter of 1995, but other launch dates are delayed by up to six months. Man-tended capability slips from the fourth quarter of 1995 to the first quarter of 1996 and permanent-manned capability slips from late 1996 to late 1997. The launches of the Japanese and European modules, due in late 1997 would also slip by up to six months.

Some consolation for the delay is that NASA will have more time to tackle some of the technical hurdles still to be overcome. The NRC committee says that the plan for advanced automation and robotics is "too small to address the magnitude of problems that the Space Station will likely encounter". It also warns NASA of the consequences of not paying more attention to the standardization of equipment. The design of the space station, it said, is becoming increasingly complex because of congressional directives, the shuttle's limitations and annual budget uncertainties, which are causing "schedule and programmatic turbulence".

Christine McGourty

HIPPARCOS

Original mission abandoned

Munich

THE European Space Agency (ESA) gave up hope last week of bringing the astronomy satellite Hipparcos into its intended orbit. Instead, project leaders were to sign an agreement on 4 September to try to salvage the mission by raising slightly the current elliptical orbit. They expect to begin this by 7 or 8 September. The new orbit would yield a sharply reduced amount of data.

The Hipparcos mission, named after the second century BC Greek astronomer who drew up the first star catalogue, was intended to measure the precise positions, parallaxes and proper motions of stars. After a perfect launch on 8 August, ESA was unable to fire the apogee motor in order to raise the \$360-million satellite into a geostationary orbit 36,000 km from the Earth (see *Nature* 340, 491 and 581; 17 and 24 August 1989).

If the rescue mission succeeds, Hipparcos is expected to last just six months before its solar panels succumb to the bombardment of protons and electrons in the Van Allen belts, 5,000 km up. This decrease from the original 30-month mission would sharply reduce the accuracy of the data that could be collected.

The salvage operation will attempt to use the hydrazine thrusters on the satellite to boost its perigee (closest approach to Earth) from 208 km to 460 km. This would reduce aerodynamic drag on the satellite and, researchers hope, give it a longer lifetime.

Raising the orbit does not guarantee that even the reduced mission can be completed. Beatrice Lacoste of ESA said last week that deployment of the solar arrays, baffles and the antennas are all controlled by motors that might fail.

The cause of the apogee motor failure is still unknown, despite five diagnostic tests carried out up to 25 August. The 500-kg motor is one of the few systems on board that does not have a back-up. A commission of inquiry was appointed last week to investigate the failure.

Another danger point will occur in January, when the Earth will pass between the satellite and the Sun, blocking the light for more than an hour. The satellite was not designed with such an occultation in mind, said Lacoste. ESA researchers are trying to find a way to prevent a power failure during the blackout.

The powerful ESA scientific programme committee will meet on 18 September to discuss whether to build a second satellite. The question is important, because a new satellite would cost £114 million (\$182 million). ESA could ask the 13 member states for the money, or the committee could reapportion it out of the existing science programme budget.

Steven Dickman

New projects flood Japan

Tokyo

JAPAN's ministries and agencies last week bombarded the Ministry of Finance with new projects related to research on the global environment in their budget proposals for the next fiscal year. Several of the requests are to establish new research centres, the biggest of which will be a new institute financed by the Ministry of International Trade and Industry (MITI) to develop "environment-friendly" technology.

The budget will not be finalized until the end of this year but requests related to science and technology usually undergo only minor adjustments in the end-of-year negotiations.

MITI has asked for ¥8,540 million (\$60 million) to set up the 'Industrial Technology Research Institute on the Global Environment', an enormous request for the first year of a new project. MITI hopes to plough about ¥2,000 million of this into development of substitutes for chlorofluorocarbons and into methods of converting carbon dioxide into useful products. Both chemical and biological carbon-dioxide fixation will be studied, according to Hiroshi Asahi, deputy director of MITI's Environmental Protection Division.

The new institute will also develop biodegradable plastics and use biological reactions to produce useful chemical materials, Asahi says. He expects the institute to be staffed by about 50 researchers from industry and MITI's national research laboratories in its first year. And he says that MITI hopes industry will provide about one-third of the costs of joint development programmes which will run for three to five years.

The Environment Agency, which survives on a budget about one-twentieth that of MITI, wants almost to treble its outlays for global environment research to ¥2,400 million (\$17 million). Half of this sum will provide new funds to promote research on the global environment in universities and research institutes both in Japan and overseas. But the agency is not sure how it will channel funds to foreign researchers. The accounting is a "difficult problem", says Yasuo Takahashi, section chief of MITI's Environmental Research and Technology Division.

The agency has also requested ¥423 million to establish a new centre for global environment research at its National Institute for Environmental Studies in Tsukuba. The centre will have a permanent staff of between 10 and 15, as well as guest researchers, and agency officials hope to lease a supercomputer. The centre will concentrate on monitoring environmental problems in the Asia-Pacific region, Takahashi says.

The Science and Technology Agency

also wants to reorganize its National Research Center for Disaster Prevention in Tsukuba, to incorporate global environment research and has requested ¥194 million to do so. And the agency hopes almost to double its outlay for remote sensing of the Earth (¥4,190 million), including development of the advanced Earth Observation Satellite (ADEOS) which is scheduled for launch in the mid-1990s.

The Ministry of Foreign Affairs seems set to get the biggest percentage increases in budget with a request of ¥534,000 million, nearly 15 per cent up on last year. Most of the request is for Overseas Development Aid (ODA), a large percentage of which is earmarked for protection of the global environment. At the Paris summit in July, former Prime Minister Sousuke Uno pledged to spend ¥300,000 million (\$2,000 million) of ODA on the environment over the next three years. But Japanese ODA organizations are notoriously understaffed and lacking in environmental expertise.

The foreign ministry and the Ministry of Agriculture, Forestry and Fisheries have requested a combined total of about \$10 million for the International Tropical

Timber Organization in Yokohama. This will make Japan by far the largest contributor to the organization, which aims at promoting sustainable management of tropical rain forests. The agriculture ministry has also asked for several million dollars (¥394 million) to establish an information centre for monitoring changes in tropical rain forests using remote-sensing technology. But Japan, coming under mounting international criticism as the world's largest importer of tropical timber, has yet to take any measures to reduce its timber consumption.

Even the Ministry of Construction and the Ministry of Transport have requested small budgets to study global environment problems. The construction ministry recently released a report estimating that sea level could rise by 0.5 to 1.5 metres by 2030 if the concentrations of carbon dioxide and other greenhouse gases continue to rise at their present rate. The report calls on the government to add 4–5 metres to shore embankments as a protective measure. The transport ministry estimates a rise in sea level of 1.1 metres and says shore protection work will cost ¥3.8 million (\$26,000 million). The two ministries will carry out further studies of sea-level change and ways of reducing greenhouse gas emissions in housing and transport. **David Swinbanks**

US agencies unite on planet Earth

Washington

In an attempt to integrate the key questions concerning global change and to develop a coordinated research programme, the US administration last week released a comprehensive plan setting research priorities for the study of planet Earth. The 196-page plan's key feature is that it is a cooperative effort from all of the seven US federal agencies involved in global change research.

It is the first time that they have all sat down together, shown each other their programmes and agreed to work together to "monitor, understand and ultimately predict global change".

The plan is the work of the Committee on Earth Sciences (CES) of the Federal Coordinating Council for Science, Engineering and Technology, an interagency body led by the White House Science Advisor. In essence, it is a manual for policy makers and those who decide how grants from research agencies should be given out.

But by repackaging a variety of research programmes from different agencies, paid for from different budgets, into one whole, it will also provide a useful weapon in the fight for more funds to support global change research. President George Bush has proposed spending \$191.5 million on global change research activities in 1990, an increase of 43 per cent over the current year. According to Dallas Peck, director of

the US Geological Survey and chairman of the Committee on Earth Sciences, Congress so far appears willing to meet the president's request, and in some cases is providing "an embarrassment of riches." Substantial increases in expenditure are planned further into the future.

"Our Changing Planet: The FY 1990 Research Plan" groups scientific tasks into seven categories and ranks them in order of priority. The study of climate and hydrological systems comes top of the list, and within that category, the role of clouds and of ocean circulation are given the highest priority of all. The two topics are the biggest unknowns in models that attempt to predict whether substantial global warming will take place. Clouds both reflect heat back into space and trap heat in the atmosphere; how the distribution of clouds will be affected by global warming is thus crucial to predicting future temperature changes. The exchange of heat between oceans and atmosphere is dependent upon patterns of ocean circulation that again may be altered by changes in global temperature.

The goal of the research programme is to distinguish between and predict the effects of natural and human-influenced changes, not to set policy on how to slow global change. That will come only after much better predictions are available.

Alun Anderson

AUSTRALIAN RESEARCH FUNDING

Quarrel over share-out

Sydney

AUSTRALIAN medical researchers are once again complaining that they have been short-changed by the Australian Research Council (ARC) and, thanks to the way ARC is interpreting a government report, will be left without funds to support their research infrastructure for the next three years.

In its May science budget, the Australian government allocated A\$107.5 million for higher education infrastructure. The allocation came in response to the Smith committee's report in March which recommended an increase in infrastructure support. The funds provide for items that are not covered by specific project funds, such as journal acquisition, animal-house maintenance and the purchase of general equipment. Medical researchers now find that they are ineligible for any part of these funds. Professor Don Aitkin, chairman of both ARC and the working party set up to decide how the funds should be distributed, says "for the ARC to provide research infrastructure to clinical medicine and dentistry would be to provide a certain amount of 'double-dipping'. The National Health and Medical Research Council (NH & MRC) received a separate allocation of \$25 million to be spent over three years on "public health, training and equipment" in the May budget. Medical researchers had understood this money to be earmarked for new

UK RESEARCH COUNCILS

New secretary for NERC

London

DR EILEEN Buttle will become secretary of Britain's Natural Environment Research Council (NERC) on 18 September. Buttle,



who leaves the Ministry of Agriculture, Fisheries and Food (MAFF) where she is head of the Conservation Policy Division, was Establishment Officer at NERC from 1985 to 1988. She has a PhD for study in marine zooplankton, has worked at the Pig Industry Development Authority and the National Institute for Research in Dairying, MAFF and has joined the Civil Service Senior Professional Administrative Training Scheme.

Ben Webb

projects and scholarships; ARC believes it should be used to maintain research infrastructure.

"If we did use the money for infrastructure, then we would have a static budget allocation over the next three years, which makes no sense", said Professor John Coghlan, chairperson of the Medical Research Committee of NH & MRC, and deputy vice-chancellor (research) at the University of Melbourne. "The working party misunderstood the [May] budget statement".

Medical researchers' problems are compounded by an earlier budget redistribution. Last year, the Minister for Education, John Dawkins, announced that A\$125 million would be pulled out of the universities over three years and distributed competitively by ARC. The clawback, as it is termed, was to be taken out of operating grants. According to Professor Ian McLoskey, head of the faculty of medicine research committee at the University of New South Wales, "the money was taken out of operating grants and redistributed as project grants. When competing for project grants, it must be specified that infrastructure funding is already available. Where will that money come from?"

The Smith committee advised that medical research, which makes up 20 per cent of all research at universities, should receive 20 per cent of the clawback as competitive grants. ARC decided, however, to release only 10 per cent for medical research. While researchers were unhappy with this deal, worse was to come.

ARC interpreted the Smith committee directive to mean 10 per cent of the increase in funds, instead of 10 per cent of the total; this means that in 1990, when clawback funds go up from A\$20 million to A\$40 million, medical researchers will receive only A\$2 million, rather than the A\$4 million they expected.

According to Professor McLoskey, the A\$2 million shortfall "accounts for 50 new projects a year out of a total of 750. This A\$2 million is likely to hit the little battlers of medical research rather than block grants for institutions." There has long been tension between medical researchers and ARC. Medical researchers resent having their funding determined by a committee which grants them no representation. According to Professor David Penington, vice-chancellor of the University of Melbourne, the ARC's agenda is to reduce funding for high-profile areas such as medicine. "Professor Aitkin holds the belief that medicine has done well enough for long enough and, in the name of equity, they must give up some of their money to less high-prestige areas", he said.

Tania Ewing

MOUSE BREEDING

Jackson Lab should stand on its own feet

Washington

THE US National Institutes of Health (NIH) have indicated that they will not gladly accept money to reestablish the colonies of genetically inbred mice that were wiped out during the fire at Jackson Laboratory in Bar Harbor, Maine, in May (see *Nature* 339, 169; 1989).

Before its August recess, the Senate passed legislation introduced by Senator Edward Kennedy (Democrat-Massachusetts) authorizing a \$25 million grant for the construction of a laboratory mouse-breeding facility, to be administered by NIH.

But NIH contend that as a private company, Jackson Laboratory should use its insurance money and other resources to recover on its own, without federal assistance. A policy statement prepared by NIH says that even if Congress allocates money for a facility to NIH, "the agency has a number of pressing needs for which the funds could be used". Breeding stock for the 1,700 strains of mice bred at Jackson Laboratory were saved from the fire; the company hopes to achieve half its production capacity before the fire within the next six months.

Carol Ezzell

SATELLITES

First commercial launch in US

Washington

THE successful launch on 27 August of Marcopolo 1, a British communications satellite that will transmit television programming for British Satellite Broadcasting Ltd from a geostationary orbit above the Atlantic Ocean, marked the entry of McDonnell Douglas, the US aerospace corporation, into the field of commercial rocketry. Marcopolo 1, launched by a Delta 187 rocket from Cape Canaveral, went into space ahead of India's Insat 1-D, which was damaged on the launch-pad in June (see *Nature* 339, 649; 1989). The Indian satellite is under repair, and may now be ready for launch next spring.

McDonnell Douglas has been building rockets for the National Aeronautics and Space Administration (NASA) for decades. Its entry into commercial space activities was prompted by the Challenger shuttle explosion of 1986, which rendered NASA, until then responsible for all payload and manned launches, temporarily impotent. President Reagan then directed the US Air Force to put satellites into orbit using its fleet of expendable launch vehicles (ELVs), and also encouraged ELV manufacturers to offer their services commercially. McDonnell Douglas, along with Martin Marietta and General Dynamics, has dozens of commercial launches planned for the next few years.

David Lindley

About-turn on regulations

Washington

IN response to thousands of complaints about its original proposals to regulate the welfare of laboratory animals, the US Department of Agriculture has made a dramatic about-turn in its approach to the issue, conceding that research institutions should themselves decide how to meet the required standards.

But animal welfare groups are angry that almost four years after Congress passed the legislation requiring the new regulations, the result is 'performance-based' standards which will be difficult to enforce and, they say, may not improve the welfare of laboratory animals.

Parts one and two of the new regulations, developed by the Animal and Plant Health Inspectorate Service (APHIS), were published in their final form last week. Significant revisions from the original plans bring the regulations closely in line with the current Public Health Service guidelines. Dale Schwindeman of APHIS says that institutions already following those guidelines will have to make few changes to meet the new regulations.

The third and most controversial part of the regulations, which includes the standards for cage sizes, exercise for dogs and means for creating environments for primates in order to improve their 'psychological well-being', is being drastically revised in response to the 18,000 comments received since it was first published in March. Schwindeman says the final rule will not be ready before the end of the year.

Animal-welfare groups have won some small victories. The institutional committees which will monitor compliance with the regulations, with the power to suspend research immediately, must include one member of the public and to investigate complaints from the public. That the regulations acknowledge public opinion at all is "fantastic", says Bob Cotreau of the Animal Welfare Institute. He also applauds the fact that the regulations define pain according to human standards.

Despite strong opposition from researchers, APHIS insists that inspectors should have full access to research facilities with authority to take photographs and photocopy relevant documents.

It argues that the inspections would not disrupt research, and that documents would not be available to the public. Neither did researchers succeed in persuading APHIS that disclosing the location of facilities where animals are housed would create security problems.

APHIS originally estimated the costs of implementing the regulations to be about \$900,000 million, but the ultimate cost will depend on the final form the standards take when they are published next year.

Christine McGourty

Abortion issue destroys board

Washington

THE US Congressional Bioethics Board seems certain to be dissolved next month after four years in which it has made no progress on any of its planned studies on controversial ethical issues in biomedical research and health care. The board has spent most of its time arguing over the selection of members of its advisory committee, with candidates' views on the abortion issue providing the litmus test for admission.

The board is a bipartisan group of 12 congressmen established to consider issues such as the withdrawal of nourishment from terminally ill patients, genetic engineering and fetal research.

The demise of the board is ensured by the action of its newest member, Senator Don Nickles. He has introduced an amendment to the bill that provides funds for the committee, blocking further expenditure until the board succeeds in selecting a chairman, vice-chairman and fills all vacancies on its advisory committee.

The Senate is likely to support the amendment when it votes on the bill in the next few weeks, making it impossible for the board to continue to function unless it can resolve its internal problems. That now seems virtually impossible.

Earlier this year, it seemed that the committee might finally be able to begin its first study, on genetic engineering, but the death of one member opened a new vacancy and all work was suspended.

One of the casualties of the board's inactivity is a decision on federal funding of fetal research, which rests on a report due from the advisory committee. At present only fetal research which is either therapeutic or within a strict 'minimal risk' standard can be carried out. That standard allows non-invasive procedures and blood tests but prohibits any new area of fetal research. Research that exceeds the minimal risk standard requires a waiver from the Department of Health and Human Services (DHHS). But in 1985 it was decided that waivers would not be issued until a report was received from the committee on their use.

Advocates of fetal research are divided over the existence of the Congressional Bioethics Board. "We wouldn't want it to be reauthorized if nothing is going to be done", said Sarah Carr, of the Association of American Medical Colleges (AAMC). "If you're opposed to fetal research then that's a good way to keep it from happening." Critics of the board say that the legislation that created the committee was designed in such a way that there would be a stalemate on everything.

The AAMC is urging the federal government to reconstitute a separate DHHS Ethics Advisory Board (EAB)

whose charter expired in 1980. Advocates of fetal research were pleasantly surprised last year when the DHHS published a proposed charter for a new EAB (see *Nature* 334, 185; 1988), but no further action was taken and a final charter has not been published. The AAMC recently wrote to the assistant secretary for health, James Mason, saying that "the absence of a board since 1980 has inhibited progress in all research involving human *in vitro* fertilization as well as research on the prenatal transmission of AIDS and on new diagnostic imaging techniques".

John Fletcher, professor of biomedical ethics and religious studies at the University of Virginia said there are signs that the reformation of the EAB "is being taken very seriously".

"Reproductive biology in the federal sector of science is being suppressed by the absence of the EAB... the US is way behind the United Kingdom in the study of the causes and treatment of infertility", he said.

The AAMC is also urging the Secretary for Health, Louis Sullivan, to lift a ban imposed last year on the use of fetal tissue in research. At that time the assistant secretary for health requested a report on the issue from the National Institutes of Health.

The report concluded that the use of aborted fetal tissue in research is acceptable provided strict safeguards are followed to prevent potential abuses (see *Nature* 339, 411; 4 June 1989). No action has been taken in response to the report, which now lies with Mason.

Christine McGourty



This 8-foot-diameter astronomical clock was installed this summer at Leicester University. The astrolabe projection shows 'clock' time as GMT (the white fleur-de-lis), as well as sidereal and solar time, relative positions of Earth, Sun, Moon and stars, phases of the Moon and rising and setting over the local horizon. The clock was designed and built by Alan Mills and Ralph Jefferson.

Dispute over monitoring

London

A DISPUTE broke out in the United Kingdom last week over responsibility for monitoring the release of genetically engineered organisms into the environment. The British government proposed recently that a new body should be responsible for environmental safety. But the Health and Safety Executive (HSE), which has the responsibility now, and the Confederation of British Industry (CBI), criticized the government's plan.

The HSE's Advisory Committee on Genetic Manipulation (ACGM) bases its decisions on releases on legislation in the Health and Safety at Work Act, but its remit does not stretch to ensuring environmental safety. The Department of the Environment, in a green paper (consultative document) outlining legislation for this autumn's 'Green Bill', revealed plans to plug this gap with a new body. And this week the HSE agreed that "the absence of legal powers in the area of environmental safety must be addressed". But it warns against splitting the regulatory system between environment and the workplace and recommends the formation of a new committee under the auspices of the ACGM to advise the HSE and ministers on release proposals.

Brian Richards, chairman of the CBI's Biotechnology Working Party, also said a new body would be a mistake. "The ACGM has been doing a good job for years, and should be allowed to continue its work, strengthened to cover environmental issues", he said. "There is too

much at stake to risk the introduction of sub-standard regulations." The Royal Commission on Environmental Pollution (RCEP) also recommended that the ACGM should be constituted in its own right to advise the Secretary of State for the Environment and the HSE on each proposed release. In a report that took three years to complete, the RCEP praised the committee and suggested it should also produce a code of practice, advise on the need for research and the possibility of categorizing new releases, and produce an annual report. The HSE also called for further discussion of the definition of genetic manipulation, the balance between protection of commercial confidentiality and public information and controls on products consisting of genetically manipulated organisms.

The Department of the Environment said the alternative proposals would be considered before the bill goes before parliament. The criticisms of the government proposals coincide with the announcement by the Department of Trade and Industry of a £1.3 million, three-year research programme into the "fate" of genetically manipulated organisms in the environment. The programme, called Prosamo (Planned Release of Selected and Modified Organisms), will investigate "new methods for detecting the spread and survival of very low numbers of modified organisms so that the results of releasing new products into complex natural environments can be assessed more accurately".

Ben Webb

HUMAN FRONTIERS PROGRAM

Call for grant applications

Tokyo

JAPAN last week launched its long-awaited Human Frontier Science Program with a call for applications for grants, fellowships and subsidies for international workshops (see page 17 of 'Classified' in *Nature* of 31 August).

Twenty three-year grants worth about \$500,000 a year will be awarded to international teams of scientists led by principal researchers from one of the seven 'summit' nations or non-summit European Community nations for research on the brain and biological functions at the molecular level. In addition, the programme will support about 150 long- and short-term fellowships and 10 workshops for research in these fields.

Conditions for the grant applications are similar to those for a 'pilot' programme launched last year, except that the research teams do not need to include Japanese. The pilot programme has come under some criticism from Western scientists whose

applications failed because they received no explanation for failure (*Nature* 340, 494; 1989).

The guidebook for this year's grant applications retains a clause stating that "the review process will be confidential; no explanation, therefore, will be offered and no correspondence can be entered into on decisions taken". But Toichi Sakata, director of Frontiers at the Science and Technology Agency, says that once the Frontiers foundation is established in Strasbourg next month, the Frontiers science council and/or grant review committees can alter this rule if they think appropriate.

Last week, the agency and the Ministry of International Trade and Industry (MITI) applied for a total of ¥3,307 million (\$23 million) for Frontiers in 1990. And with the increased budget and some support from other nations, Sakata hopes the number of grants will increase to "about 26 or 27" in 1990.

David Swinbanks

INSERM's man speaks out

Paris

IN a book due to be published shortly, the director-general of the French national institute of health and medical research (INSERM), Philippe Lazar, suggests that major journals inadvertently run the risk of discouraging originality in research. In a chapter entitled 'Avatars of originality', Lazar criticizes *Nature* over its reactions to the controversial paper by Jacques Benveniste on the molecular 'memory of water' (see *Nature* 340, 178; 1989).

In his book, which gives the reader an 'inside view' of the preoccupations of the director of a national medical research organization, Lazar defends INSERM's practice of four-yearly laboratory reviews by an elected committee of experts. The system, he says, should maximize the emergence of "truly innovative work" because, for laboratories wholly funded by INSERM, the reviews are retrospective. But, says Lazar, research which is "off the beaten track" stands little chance of being published quickly, both because it is usually unproductive at first and because major journals may be reluctant to publish it. And publication is often a criterion of merit when assessing grant proposals.

By publishing Benveniste's paper on condition that the experiments be repeated in the presence of a panel sent by *Nature*, Lazar says that the journal cast itself in the role of scientific "establishment". He wonders whether *Nature*'s editors "had a real moment of difficulty at the idea of challenging, in their own right . . ." an example of "truly original research".

Nature, he adds, should have left it to the scientific community "to play its fundamental role of filter at its own pace". Any other procedure, he says, is "dangerous" and evokes "the spectre of 'official' science".

These are substantially the criticisms made by Lazar at a press conference last month at which it was announced that Benveniste's tenure had been renewed until the end of the year.

Lazar also attacks the press for what he considers an excessive zeal during the height of the affair.

To counterbalance the weight accorded by grant review committees to publication in major journals — and the consequent influence of conservative peer reviewers on the kinds of research supported — Lazar wants to offer a series of small grants for 'high risk' innovative research. He says he is not convinced the idea will work, but adds that he would "regret having to renounce the idea for want of support".

Peter Coles

Les Explorateurs de la Santé is published by Editions Odile Jacob, Paris, later this year.

Campaign to close Semipalatinsk

Moscow

A MOVEMENT whose goal is a moratorium on nuclear testing by the United States and the Soviet Union is making some headway. Last month, the organization, called the Nevada-Semipalatinsk Anti-Nuclear Public Movement, called on presidents Mikhail Gorbachev and George Bush to put an end to underground weapons tests.

The president of the movement, the prominent Soviet Kazakh poet Olzhas Suleiminov, who is also now a People's Deputy of the USSR, says that the "immediate goal is to close the testing-ground in Semipalatinsk (in Kazakhstan). But Suleiminov added that the goal will be attainable only if there is "simultaneous closure of the Nevada testing ground".

The movement was founded at the end of February this year, with a rally at Alma Ata, the capital of Soviet Kazakhstan. Its general aim is to put an end to nuclear testing anywhere in the world. The organization is hoping to pool its efforts with those of similar groups in the United States, for which purpose a member of the movement's coordinating council has visited the United States, meeting among others Dr Bernard Lown, co-chairman of International Physicians for the Prevention of Nuclear War.

The Nevada-Semipalatinsk movement accuses the Soviet authorities of concealing the consequences of the testing programme in East Kazakhstan. The group's formal statement says that both ill-health and mortality increased in the neighbourhood of the testing-grounds over the forty years they have been in use, adding that "this was concealed from us". It complains that problems occasioned for public health and the environment during the testing programme were neglected.

The report of an expert commission formed on the instructions of the Soviet government, which concludes that the radiation in Semipalatinsk and surrounding districts is normal, has been met — mildly speaking — with open mistrust by the local people. The letters with which state and public offices have since been deluged have been full of tales of suffering and sorrow.

E.I. Proskurina, born in Semipalatinsk in 1936, says that "everything that once made me a woman was cut out, and I do not know who I am now". Zh. Zhunishanov describes having seen "with our own eyes enormous blinding glows which turned into mushroom clouds ... the gullibility was so great that we did not feel the danger and did not attach any importance to these sights".

Among the letters is one from 20 war and labour veterans who say they were, like others in the Abai district, subjected

to radiation from the testing of a hydrogen bomb in 1953. In the rural area of Sarzhai, 240 people are under medical supervision. Cancer, anaemia, allergy and neurosis are common, child mortality is high and suicides among young people have become more frequent.

Although there have been only underground tests since 1963, they have caused repeated emissions of radioactive gases, most recently on 12 February, and background radiation has been increasing. The effects of seismic stress are another source of trouble. A public opinion poll carried out in May and June showed that 99 per

RADIATION SAFETY

Byelorussian criticism

London

THE Byelorussian Academy of Sciences is highly critical of the standards of radiation safety that were proposed by Moscow experts in the aftermath of the Chernobyl disaster, the president of the academy, Dr Uladzimir Platonau, told a recent session of the Byelorussian Supreme Soviet (parliament).

The Moscow experts from the Ministry of Health Protection and the All-Union Academy of Medical Sciences set a maximum lifetime radiation dose of 35 rems. People living in regions of greater risk are to be evacuated. But the caesium-137 pollution from Chernobyl is proving more extensive and far more difficult to remove than was originally estimated, and more than 103,000 inhabitants of the Byelorussian republic now face evacuation. Nevertheless, say the Byelorussian scientists, this limit is not only scientifically doubtful but is also "amoral". How, Platonau asked his fellow parliamentarians, can one have the same, artificial limit for adults, children, pregnant women and invalids? The real criterion, according to the academy, Platonau said, should be whether or not normal life can be sustained in a given area.

Approximately one-fifth of all agricultural land in Byelorussia is at present unfit for agriculture, and the residents of these erstwhile collective and state farms have to be supplied with milk, meat and vegetable products from outside. The cost of this operation is so high that, according to one Byelorussian estimate, if the whole of the contaminated area were evacuated, the outlay, including building new homes, schools and amenities, would pay for itself in eight years.

Platonau's proposal is less drastic and more democratic. He suggests that a maximum lifetime dose of 7 rem (the international standard for nuclear power station construction) should be adopted as

cent of the inhabitants of three districts adjacent to Semipalatinsk felt unwell the day after an explosion, and that 99.5 per cent are convinced that the testing-ground endangers their health.

Saim Balmukhanov, a member of the Kazakhstan Academy of Sciences, says that the number of people suffering from blood diseases has doubled since 1970 and that infant mortality has risen sharply, to 43.5 deaths per 1,000 newborns. Now, in a street in central Semipalatinsk, there is a drugstore in whose window can be seen a dosimeter showing the radiation level in the city, put there at the urging of the movement that seeks a moratorium on nuclear testing.

Vladimir Lysenkov/Novosti

an evacuation threshold. People living in areas of higher risk should be given the fullest possible information about the degree of pollution and the risk factors, and should then be given the option of evacuation.

Ever since the Chernobyl accident, in April 1986, Platonau and his academy colleagues have been concerned about the consequences for Byelorussia, but have received short shrift from Moscow. Two years ago, he told the Supreme Soviet, they had put a number of questions about radiation safety standards to the Moscow experts, only to be told that "you are not medical experts, and there is no need to discuss these doubts further". It is true, Platonau said, that the Byelorussian Academy has no medical institute, and that its Institute of Radiobiology was founded only in 1987. Nevertheless, during the past year, Platonau said, he has been collecting all relevant data on radiation safety from his biologist and chemist colleagues. This material, he said, "has made it possible to draw the attention of the broad scientific community and the leaders of our country to the fact that the situation is far more complicated than certain comrades imagined". The Byelorussian concern about post-Chernobyl health risks is shared by the Ukrainian Academy of Sciences, which also queries the scientific validity of the 35-rem limit, and whose president, Dr Borys Paton, has proposed a moratorium on nuclear power construction until a safer generation of reactors is available, and the health risks better understood. Somewhat unexpectedly, the All-Union Party daily *Pravda* has shown itself to be sympathetic to the Byelorussian case, even emphasizing, for example, the shortage of diagnostic equipment to examine children with thyroid problems associated with iodine-131 fallout from Chernobyl.

Vera Rich

ALASKAN OIL SPILL

Rescued sea otters fly the coup

San Francisco

INITIAL radio transmitter data from the 193 captured sea otters which were released into Prince William Sound late last month have held several surprises for marine biologists tracking the mammals. The otters had earlier been rescued from the giant Alaskan oil spill.

Despite rough weather that disrupted signals, researchers have stayed in touch with the 45 otters carrying surgically implanted radio-transmitters. All were released north of Cordova, in the eastern part of Prince William Sound and away from the devastating effects of the March spill of some 10 million gallons of crude oil. The slick drifted west into the Kenai Peninsula area, where most of the captured otters were rescued.

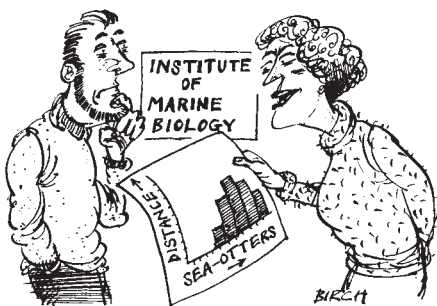
So far, however, it seems that none of the otters has returned home. "Our best information is that the otters are all still in the sound", says Tony DeGange, a wildlife biologist with the US Fish and Wildlife Service.

They were thought likely to move back to the Kenai, where they were caught. But most have dispersed from the release point, and a few have already travelled several hundred kilometres.

Wandering is expected, especially among females, which have large seasonal movements compared to the more territorial males.

Researchers next plan to install radiotags on other otters that remained free

They must be looking for an Exxon representative...



throughout the crisis. Some will come from the oil-spill zone, the remainder will be a control group from untainted eastern waters. The idea is to "validate the whole rehabilitation process", says DeGange, and to see if the otters that were treated and released "become full-functioning members of the sea otter community again". The free-ranging otters from the spill area will be compared with the control group to see if researchers can detect any effects—in survival and reproductive rates—of living in the oil zone.

Robert Buder

UK ATOMIC ENERGY

Slimming down for sell-off

London

THE United Kingdom Atomic Energy Authority (AEA) is to undergo a rigorous rationalization programme aimed at attracting finance from the private sector after a series of cuts in government funding. The authority will split its business activities into nine discrete areas, each covering a sector of research which is considered commercially viable. John Collier, chairman of AEA, revealed last week that nine chief executives would head the subsidiaries independently under the authority's new commercial banner, AEA Technology.

The nine business areas are the fast reactor, fusion technology, nuclear fuel cycle technology, environmental protection services, thermal reactors, contract research, oil and gas, risk management technology and decommissioning and radioactive waste management.

The AEA had incurred inevitable restructuring costs and had to reduce manpower to fit estimated future turnover and compensate for the reduced government contribution to research into the fast

reactor, according to Collier. These costs, with a £27 million provision for the future, left the authority with a current loss of £41 million for 1988–89.

Collier criticized government cuts in support for the fast reactor and energy research programmes. "To be serious about nuclear power requires us also to be serious about the fast reactor", he said. The government has also warned that it plans to cut support for the Dounreay fast reactor and fusion work at Culham.

But Collier introduced a note of optimism when he revealed that the authority had signed a series of agreements in Bonn to ensure British involvement in the future of European research into fast reactors. He said the deal provided a "sound basis" for the AEA to move to the "demonstration phase" of fast reactor development with partners in France and West Germany.

"The European collaboration is the best and perhaps only way for the UK to ensure fast reactors are available when we need them early next century", he said.

Ben Webb

EARTH SCIENCES

Germans to drill to 10 km

Munich

THE West German government announced last week that it will continue to pour money down a hole in northern Bavaria. The money, DM500 million in all (\$256 million), is to be spent on what was meant to be the deepest hole in the world. Now the hole will go only 10 km down, but it will still give geologists a glimpse of an unexplored part of the Earth's crust.

The decision to go ahead with the project, officially known as the "Continental Deep-Drilling" project (KTB in German), was based in large part on the success of a 4,001-metre-deep pilot hole drilled near the Bavarian town of Windischeschenbach. Researchers will begin drilling the deeper hole in August 1990. They hope to complete it in 1994.

The West German geology community strongly supports KTB, which, along with the Soviet deep hole on the Kola Peninsula, is one of the most ambitious deep-drilling projects in the world. The West German Research Ministry (BMFT) has also been generous in its support for KTB. The latest budget includes an increase of DM50 million above the original figures. But a BMFT spokesman stressed that the ministry was backing KTB entirely for scientific reasons, and not, as some have speculated, because of the good publicity the project has been generating.

Plans to drill to 14 km had to be abandoned when the pilot hole encoun-

tered the unexpectedly high temperature of 118°C at 3.5 km. The temperature will probably climb to the functional limit of the equipment—300°C—by the time the hole reaches 10 km. If it is less hot, the researchers will decide in 1993 if they want to try to drill down to 12 km. Ironically, the drilling site was chosen because the crust was expected to be cooler than at other sites in West Germany.

And there have been other surprises. The cores brought up from 3 km were under extreme stresses that researchers had expected to find only at 7 km or deeper. At 3.4 km, the drill-bit hit fissures filled with salty brine that may be a remnant of primordial oceans.

Below 3.2 km, large amounts of helium and methane were found. The methane may derive from the crust itself, rather than organic matter on the surface, lending support to those, such as astronomer Thomas Gold, who claim that natural gas can be found in the crust. Gold has led a deep-drilling project in Sweden intended to find useful amounts of methane gas in crystalline rock, where it has never been found before.

The KTB team has also made advances in drilling technology. For instance, they have developed a special 'drilling mud' a colloidal silicate pumped into the hole to lubricate the rotating diamond drill-bit which oozes up the hole, shoring up the walls.

Steven Dickman

More on animal experiments

SIR—Clive Hollands is to be commended for raising important issues regarding the responsibilities of scientific journals to uphold ethical standards regarding animal experimentation. (*Nature* 339, 248; 1989). Hollands specifically objected to *Nature's* publication of experiments, conducted in France, involving major surgery with anaesthesia on two pregnant macaque monkeys to remove bilaterally the eyes of two fetuses (*Nature* 337, 265–267; 1989). The fetuses were returned to the uterus and the pregnancies later terminated by caesarean section. The young monkeys were allowed to survive for some weeks and were then killed for tests to assess the influence of retinal input on cortical development. "Such gross interference with a highly intelligent species requires overwhelming justification — if it can be justified", says Hollands. He considered these experiments would not be legal in the United Kingdom.

A subsequent leading article responded by stating that *Nature's* "rule of thumb" has been "that the results of animal experiments that would not easily win general regulatory consent had better be of exceptional interest if they are to be published" (*Nature* 339, 324; 1989). On two grounds, this is an inadequate response.

First, *Nature* gives no evidence that the criterion of "exceptional interest" is explicitly taken into account. Both the significance of the results and the trauma to the animal have to be identified in order to weigh the justification for conducting the experiments. The exceptional interest of retinal input on cortical development should not be assumed to be apparent to all readers.

Second, *Nature's* "rule of thumb" does not incorporate the concept that some procedures are beyond the pale irrespective of possible scientific merit. As Patrick Bateson, professor of ethology at the University of Cambridge, speaking for the animal ethical review committee of the journal *Animal Behaviour* has said, "If suffering was likely to be intense, we would not regard work as acceptable and would recommend rejection (for publication) even if the work was of high standard", (personal communication, 28 October 1985). He goes on to say: "Intermediate levels of suffering would be justified by high quality work. Low quality work would not justify any level of suffering and, in any event, would not be published in the journal anyway."

So how should journals handle manuscripts submitted for publication that appear to raise serious ethical issues? Should journals merely act as "passive entities", as John Maddox describes, leaving it up to the scientific community to

make subsequent judgement (*Nature* 339, 657; 1989)? Or should journals assume an active role in helping to uphold high ethical standards? I believe the latter is the responsible course.

Two pioneering journals have established commendable procedures for handling questionable animal experiments and have published meaningful and detailed policies on animal experimentation which appear in instructions to authors (*Pain* 12, 199; 1982 and *Animal Behaviour* 34, 315–318; 1986). The journals' manuscript reviewers are specifically charged with ensuring their compliance, and manuscripts identified as involving questionable animal experiments are referred to special committees for determination.

I do not have personal experience of serving on such a committee, but it seems to me that they could decide on one of several courses depending on the seriousness of the concern:

- (1) reject the manuscript on humane grounds,
- (2) require modification of the manuscript by the investigator for instance, to include any or all of the following: justification of the importance of the work; a description of the severity and duration of animal pain; a description of the procedures taken to alleviate the animals' suffering or
- (3) publish the article with an editor's note drawing attention to the concerns (see, for instance, the editorial note that accompanied an article in *Animal Behaviour* 32, 293–294; 1984).

Publication of an article suggests scientific approval and tends to legitimize the work. It should be reserved for meritorious work that complies with the highest ethical standards of the day.

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A creative force?

SIR—I am a scientist by training. I believe that an understanding of the Universe can be obtained only through careful observation. Models that allow us to comprehend observable phenomena can be constructed, but only when a model has been tested experimentally can it be held as truth. Nevertheless, I find myself flirting with explanations to problems that cannot be tested, knowing full well that until they can, I cannot allow myself to 'believe' in them as possible representations of truth.

My 'flirtation with the untestable' falls into two categories: phenomena that are occurring outside our time frame (such as the developmental diversification of life forms on Earth), and phenomena that

occur within our time frame but seem to involve processes for which only the results are physically observable (such as 'human intelligence' and biological 'drive', and the 'experience' of them by the individual). I have no illusion that this flirtation with the untestable is anything other than religion, because the explanations my mind comes up with invariably involve an unknown, creative force. For a scientist, this is heresy. I should not be seeking to comprehend the Universe with untestable theories that postulate the existence of an unobservable 'force'. Untestable theories in science (such as the evolution of life through random diversification followed by natural selection), are permissible, but not if they involve forces from an unknowable source.

Ultimately, when I hold such beliefs, I must give up my scientist's persona and put on a religious one. For although I continue to use scientific truth to formulate a higher-resolution picture of the knowable Universe, I still find myself groping at 5,000-year-old untestable models. Instead of Adam and Eve, my creation myth begins in a 'primordial soup' with 'self-replicating molecules'. My struggle with the mystery of human consciousness uses terms such as 'mind-brain problem' and 'immanence illusion' rather than 'soul' or 'God'. I think in terms of 'creative and degradative' forces rather than forces of 'good and evil'. But when faced with the essential question — is there a creative force manifesting itself in the observable Universe? — I can answer yes, not with irreverence to any law of physics, but rather out of a sense of awe, in the face of utter bemusement.

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Nuclear power

SIR—Although I was a signatory of the Greenpeace declaration on global warming, I nevertheless agree with Max Beran's strictures (*Nature* 340, 336; 1989). The text reached me about a week before it was due to be published and I immediately rang Greenpeace to say that I should be glad to sign but asked them to change "irrelevant" to something like "hardly relevant" or "little relevant". I was told that it was too late to make changes and I then signed. I should have declined, although I agreed with the declaration as a whole. Scientists should avoid using sweeping or emotional terms they cannot justify, even if they do so to correct a false and irresponsible ministerial statement.

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The peripatetic fossils: part 2

Vishwa Jit Gupta

On 20 April 1989 *Nature* published an article by Dr John Talent accusing "one Indian scientist" of corrupting the palaeontological literature on the Himalayas. Below, that scientist — Professor V. J. Gupta — replies. On pp.13–16 appear four other articles, each of them prompted by the appearance in print of Talent's allegations.

JOHN Talent¹ has claimed that fossil work in the Himalayas is completely unreliable because I salted rocks with specimens bought in Europe and Africa. In doing so he implicitly attacks anyone who has worked with me and dismisses out of hand the vast amount of Himalayan research by many other authorities. In none of his publications or statements on the subject has Talent¹⁻³ referred to the meeting in Calgary in 1987, when I made an open offer to the audience to visit me at Chandigarh and discuss my research — indeed, it seems he has not recently examined any of the relevant fossil collections.

Talent's attack rests on four assertions, derived mainly from the literature and anecdote, not scientific enquiry: (i) the fossils reported by me are from inaccessible areas; (ii) those fossils are anomalous and incompatible with known models; (iii) lithologies described by me are at variance with those described by others; and (iv) I have duped my co-authors the world over.

Tagging the name of one person to the fossil collections Talent refers to is gravely misleading. It is seldom possible to do fieldwork in the Himalayas by oneself. The collections were made by various teams of geoscientists.

For example, collections from Ladakh were made by members of the Manali-Leh Expedition (1969)⁴ which included eight geologists (V.J. Gupta, G. Mahajan, S. Kumar, S. Chadha, P.C. Bisaria, N.S. Viridi, N. Kochhar and S.R. Kashyap). The collections from the upper Spiti valley were made by S.P. Jain and V.J. Gupta (Lahul-Spiti Expedition, 1965)⁵ whereas collections from Kaurig in Kinnaur and parts of lower Spiti were made by a field party (Lower Spiti Expedition, 1966)⁶ which included three palaeontologists (S.B. Bhatia, V.J. Gupta and S.P. Jain) and a structural geologist (R.C. Kanwar). Collections from the north-eastern Kumaun were made by the members of the Kalapani-Kuti Expedition (1971)^{7,8} to the north-eastern Kumaun organized by the Wadia Institute of Himalayan Geology, including K.S. Valdiya, K.B. Powar, D.M. Banerjee, V.J. Gupta, S. Venkatachalam and C.M. Khanna. Field work in Nepal was done jointly with V.S. Chhetri, Senior Geologist of the Nepal Geological Survey, Kathmandu⁹.

In addition, collections were made from time to time by myself, my post-graduate students and research scholars working in

different parts of the Himalayas. At times collections were also made by geologists from other organizations and submitted to us for joint work. Some of the collections were even received from foreign geoscientists. Several papers were published in different scientific journals on the basis of preliminary field observations under joint authorship with the members participating in different expeditions or field works⁴⁻⁹.

Some remote parts of the Himalayas are now served by roads, but conditions for geological field work were very bad when we started research about 25 years ago. Even with the help of porters and bearers only limited collections of rock and fossils could be brought back. This does not mean that we should doubt the veracity of the resulting data. Further, the government did not allow us to use detailed topographic or army maps, leading to difficulties in giving precise locations.

The so-called "faunal anomalies and incongruities" referred to by Talent^{1,2} call for explanations, not accusations. Geology (especially Himalayan geology) is a developing science where anomalies should provide food for thought. Talent's conclusion of fraud³ is in part a consequence of over-hasty analysis. To help justify his cause he dismisses the value of various scientific journals, presumably because they are Indian. This no doubt, in Talent's opinion, is the failing of *Contributions to Himalayan Geology* which has attracted international contributors and subscriptions. It is a publication India can be proud of — but that to Talent is a contradiction in terms.

This close similarity of faunas recorded from different stratigraphical horizons of the Himalayas with those of other regions, their mode of preservation and the lithology of the rocks (including matrix accompanying the fossil specimens) from which they were collected have been commented upon in the original publications. The apparently controversial find of graptolites was first reported by myself and M. R. Sahni in 1964^{10,11} and has been corroborated by subsequent workers¹²⁻¹⁴. Talent ignores these papers. Similarly, the discovery of fossils from the Muth Quartzite has been confirmed by several workers¹⁵⁻¹⁷. The graptolites and other fossils from the Muth Quartzite were examined by Sir C. J. Stubblefield, at that time Director of the Geological Survey of Great Britain.

The late Professor M. R. Sahni, under whose guidance the work was carried out, himself visited the fossil localities in Kashmir in October 1964.

The Muth Quartzite is a time-transgressive sequence and there is nothing contradictory in its lower and upper boundaries straddling the upper Lower and Upper Devonian. As such, these findings substantiate my impression of its varying ages in different sections. The sequence lying below the Muth Quartzite exposed in different parts of Spiti and Kinnaur (Takeche, Gechang, Muth-Shian, Leo and Manchap) has been investigated in detail and Silurian age has been assigned to the reef buildups at these localities¹⁸. Among the fossils recorded from the reef buildups are corals, stromatoporids, solenoporids and so on. The Silurian reefoidal sequence at most of these localities is overlain by arenaceous rocks enclosing a few tabulate corals and *Psilophyton*¹⁸.

The remarkable similarity of the Devonian conodont fauna from the Himalayas to that from other areas was pointed out independently as far back as 1967¹⁹. The close identity between the Himalayan and North Evans conodont fauna was also discussed, in 1975 (ref. 20). The Devonian conodont fauna is fairly widespread in the Himalayas and is being worked out in detail on the basis of fresh collections from measured sections. We plan to undertake geochemical analysis of the conodonts using various techniques (ICP, AAS, EDX, radio mass spectrometry for stable isotopes) and our Institute is in the process of acquiring and commissioning the sophisticated equipment involved. All these data will help in ascertaining the elemental/isotopic (ratio) composition of the conodonts. This will enable us to compare the composition of our Himalayan samples with that of identical forms from different parts of the world, including the North Evans conodont assemblage. The occurrence of Devonian conodonts corresponding to the *tringularis* zone has also been reported by independent authorities from the Dolpo region, which lies "north of Dhaulagiri" in Nepal²¹, as well as from the Phulchauki section²². The Godavari-Phulchauki section is structurally very complicated, not at all like that described by Talent^{1,2}. There is a complete succession of rocks ranging in age from Cambrian to Devonian in this section, and several lithological units are

structurally repeated.

Talent^{1,2} questions the occurrence of "dolomitic sandy limestone horizons" within the Muth sequence. What can one say? Such rocks are widely present, and no one could doubt this in the field. Dr A. D. Ahluwalia is planning to write separately on the Muth-Lipak transition of this section, as the collection referred to by Talent was made by him²² and not by me. The occurrence of sporadic beds of ochreous weathering dolomitic limestone has been recorded from the Muth Quartzite exposed in the Chumik-Marpo area of southern Zaskar in Ladakh^{23,24}, Kum-aon²⁵ and Western Nepal²⁶.

Lithological variations are likely to occur in sections where one formation grades into another. The boundary must be arbitrary, as is the case with the Muth Quartzite succession and the overlying Lipak Formation as well as its equivalents in different parts of the Himalayas. To date, no stratigraphical or structural break has been reported between the fossiliferous Early Carboniferous sequences and the underlying Muth Quartzite succession.

The close similarity of many elements in the Devonian fauna of the Himalayas and other Asiatic regions to the corresponding North American faunal elements has been noted by a number of leading workers since 1913 (refs 27–31). The Zebingyi Beds of Burma have yielded many Devonian fossils which closely resemble the fauna from the Manlius Limestone of New York and lower Monoran of Michigan^{29,30}. Many species described from the Wetwin Shales are identical to the Naples fauna of western New York³¹.

The similarity of the Devonian ammonoids from the Khimokula section to those of North Africa (Morocco, Algeria), Western Europe (Germany, Poland, Spain) and Asia (USSR, Mugodjar region of the Ural Mountains, China) was originally pointed out by the authors concerned. We were aware of the mode of preservation of our faunal assemblage. As pointed out in our paper³², Dr J. Thein (Bonn) carried out the determination, by X-ray diffraction, of the mineralogical composition of the Himalayan ammonoids. The morphological differences observed, in comparison to specimens from other regions (including Morocco), were discussed. For example, the Himalayan samples of *Discoclymenia cucullata* (v. Buch) differ from those described from North Africa³³ in their whorl section, which is slightly broader across the flanks. In this respect the Himalayan ammonoids show close resemblance to specimens described from the Ural Mountains³⁴, from which they differ somewhat in having narrower venter and the less pointed adventitious lobe. Conodonts have also been recorded from the upper units of the Muth succession in this area and more

work is needed to look into the possibility that reworking or condensation and intense folding has taken place. Poorly preserved cephalopods have been reported by other workers from the loose blocks of Muth Quartzite in the type locality (the Muth in Spiti)²⁷. Fossils with a haematite mode of preservation are not uncommon in the Himalayas. Thick beds of haematite associated with the Phulchauki Series, exposed south-east of Kathmandu, contain casts and moulds of brachiopods, cephalopods, trilobites and crinoids⁹. It must be emphasized that well-documented Silurian and Devonian successions do exist in the Himalayas, which is proved beyond doubt by field and laboratory data collected from different sections.

Lewin³ suggests that I may have received Devonian fish specimens, described from Ladakh, as a gift from Dr Zhang Miman of the Institute of Palaeontology in Beijing. I have never met Dr Zhang Miman, nor have I ever visited her institute. The possibility of obtaining the fish as a gift does not arise.

I regret that Professor W. C. Sweet was mistakenly acknowledged in my paper on the Tandi Limestone³⁵. The specimens of conodonts were in fact commented upon by Professor David L. Clark from Wisconsin. This error crept in inadvertently, and I apologize for it.

Dating rocks is a difficult business, and one in which anomalies often arise. For example, the Krol-Tal sequence of the Himalayas was for a long time thought to be Permian-Jurassic in age but is now established as Cambrian. Mistakes were certainly made over the record of different types of fossils from the Krol and Tal formations³⁶.

Let me pose this challenge to the veracity and verifiability of Dr Talent's claims.

(i) Will he defend his identification of *Linoproductus* and its alleged time range for the Krol Formation? (ii) Will he at last publish the scientific results of the large fauna he was allowed to collect and take to Australia over 15 years ago from the Bijni Tectonic Unit, Garhwal Himalaya, and other regions? (iii) Will he acknowledge that I, with another palaeontologist, published a systematic study on some fossils from the same area³⁷? Will he admit that this paper has scientific validity? (iv) Will he acknowledge that faunas I described from the Fenestella Shales of Kashmir came from the Fenestella Shales of Kashmir^{38,39}? (v) Is he willing to spend time at the Geological Survey of India and examine some of the rocks and fossils in question?

Talent¹⁻³ proclaims that I have been able to fool all of the people all of the time. The period concerned is a quarter of a century, and the number of people — all of them highly regarded palaeontologists — more than 60. The contention is unbelievable. Talent himself points out that it is impossible for one person to have a detailed knowledge of so many diverse faunal assemblages. That is precisely the reason why I collaborated with so many experts, and in doing so served geology by bringing to them fossils they could not collect personally.

John Talent has made sweeping pronouncements on Himalayan geology. Yet he is not an authority on the subject. I can only conclude that his attack on me was made for two reasons — to draw attention to himself and to deflect criticism of his own failure to contribute to Himalayan geology. □

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The peripatetic fossils: part 3

The articles printed below are by co-authors of some of the papers called into doubt by Talent. Two are by close colleagues of Gupta at the Centre of Advanced Study in Geology at Panjab University.

Upper Palaeozoic of Lahul–Spiti

A.D. Ahluwalia

I WAS a member of five expeditions in the Himalayas (1977–81) organized by the Geological Survey of India (GSI), and one from Panjab University in 1988, and have an intimate knowledge of the Lahul–Spiti region. I am now a colleague of Professor Vishwa Jit Gupta at Panjab University. Most of the doubts expressed by Talent^{1–3} are well-founded. I would like to start the process of putting the record straight by pointing out some significant anomalies in the Upper Palaeozoic of Lahul–Spiti.

Devonian

In 1978, during a GSI mapping assignment, I measured and sampled the Takche–Muth–Lipak section in the Takche nala near Losar in the Upper Spiti Valley for micropalaeontological studies⁴. A part of the sample representing dolomitic limestone (20 cm), lying 5.9m below the Muth–Lipak contact in this section, was macerated in the local GSI laboratory. I had no experience with Devonian conodonts, but the part of the residue I examined was apparently barren.

In 1980, K.J. Budurov, a reputed Bulgarian conodont expert, visited the Panjab University. I took the residue of this sample to Gupta, Budurov's host, so that it could be examined afresh by the visitor. Gupta suggested that the residue bottle should be left with him to enable him to spend some time on it before showing it to Budurov, and later Gupta pointed out the presence of numerous conodonts in the residue. I felt rather embarrassed at having initially missed the assemblage, but was happy at the 'discovery' which was jointly published with Gupta, Budurov and Kanwar^{5,6}.

On reading this year that the fossil assemblage had characteristics of the North Evans conodont fauna, and that it was considered to be anomalous for the Himalayas^{1,2}, I macerated afresh the part of the Muth dolo-

mitic limestone sample that I had kept for further work. To my utter shock, I found this sample completely barren and Talent's doubts confirmed. Colleagues in GSI, who had independently looked at this sequence in 1982–83, were also disappointed. Budurov, who had taken parts of residues of my samples from this sequence in 1980 directly from me, conveyed absolutely negative results when returning the sample tubes through Gupta in 1985.

I am thus left wondering how a rich conodont assemblage, exactly like that of North Evans, had appeared in a sample which was indeed barren. There was no reason for suspicion at that time. Yet my trust seems to have been betrayed. With hindsight, one could say that I should have remacerated the sample in 1980. It is a great embarrassment for me now to officially withdraw these reports^{5,6}.

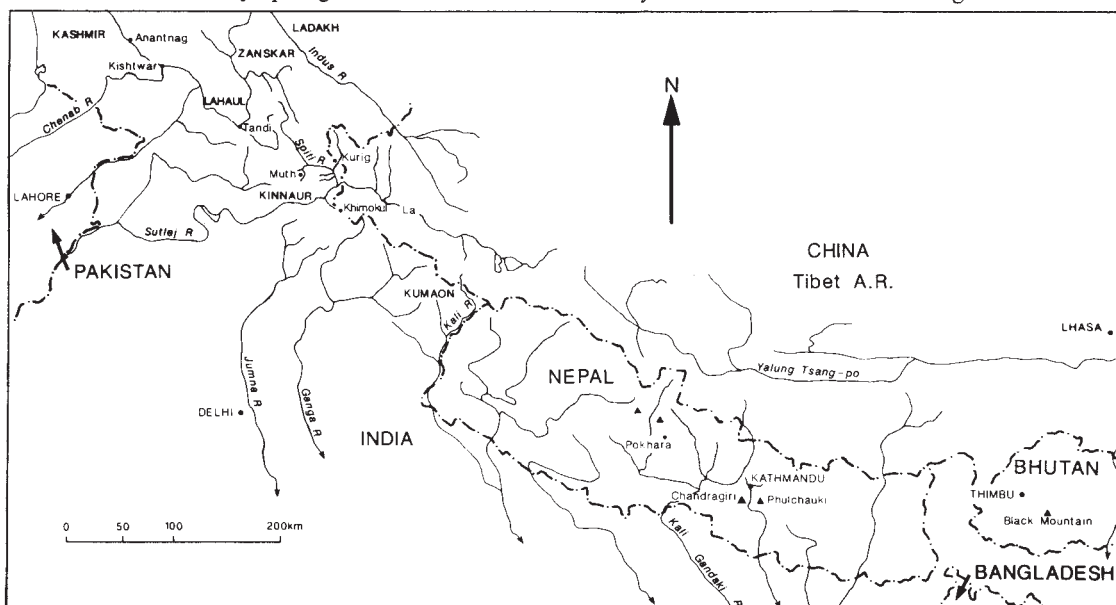
Though regionally impersistent, limestone layers are well established in the lithostratigraphy of the Takche section^{4,7}. The Muth–Lipak lithocolumn⁶ which was later borrowed and published in another of Gupta's papers⁸, is the only reliable information which survives this unfortunate episode. In view of the above, the other reports of Devonian conodonts from the Himalayas^{9–15} also become suspect. My apologies to the scientific community

for this temporary indiscretion and also to Professor A. Gansser and H.M. Bolli and their *Diplomarbeit* students at ETH Zurich, to whom I demonstrated this assemblage in 1984 with an erroneous Spiti Himalayan label.

Carboniferous

(1) In 1977, I found a well-preserved crinoid in the Lower Carboniferous Lipak Limestone at Bindi–Pahariya on the right flank of the Chandra Valley in Lahul. This was during the GSI expedition on the right side of the Chandra, downstream of Bara Lacha Ban. The crinoid-bearing part of the huge sample was taken out by Gupta, who promised to have it examined by Professor G.D. Webster. After a short span of two weeks, Gupta conveyed Webster's opinion that the crinoid was "inconsequential". He promised to dig out the letter written by Professor Webster but somehow could not. After several years, however, a description of a crinoid resembling mine, and even having a similar crack running through it, was published as from "near Surichun La" (ref. 16). My misgivings were also aroused by the presence of the Po Formation at Surichun La instead of the Lipak Formation.

(2) In 1977 Gupta had suggested to me that I should look for conodonts in the part of the samples of Lipak Limestone that I had retained. His suggestion was a good one, and I did indeed find conodonts in my samples, not only some that were Lower Carboniferous in age but also some



Principal fossil localities in the Himalayas referred to in these articles (from Talent, ref. 2).

from the Lower Triassic. I expressed my gratitude to him for his advice. But before I could report these conodonts from either area, Gupta published a note on Carboniferous conodonts from "Bara Lacha Ban" (ref. 17). He even mentioned getting similar material from Losar (Spiti) and also Kinnaur (the latter are still unpublished). Gupta was in fact told about Bara Lacha Ban in reference to Lower Triassic conodonts and he obviously seems to have got mixed up. Gupta had last crossed Bara Lacha La in 1969 with a big team¹⁶, and as late as 1975 (ref. 19) he had shown Cambro-Ordovician rocks in a geological map of this area and even Bara Lacha Ban. One can be absolutely sure that he had not been to Bara Lacha Ban, for there only the sequence from Po up to the Triassic limestone is exposed and the Lipak Formation in which he claimed to have discovered Carboniferous conodonts had been cut off by a fault^{20,21}. Gupta was a co-author, with Budurov and Kanwar, of my preliminary reports on conodonts from these two localities^{21,22}, but his own Carboniferous conodont reports now started to come from near Losar^{8,23}. He vaguely fixed the sample horizon in a lithocolumn prepared by me, but he completely ignored the fact that the Lipak Formation near Losar represents an intertidal-supratidal sequence, which has prominent evaporitic facies in parts and is not suitable for conodont occurrence. The Losar section has in fact never yielded conodonts in any of the samples checked by me, by colleagues in GSI or by Budurov. Nothing was ever heard from Budurov of our valuable conodont assemblage taken by him to Sofia in 1980 for detailed publication following the preliminary notes^{21,22,24}. Two of my Lower Carboniferous conodonts that were given to Budurov, however, appeared in a paper by Gupta²³, who stated that they came from Losar and not from Bindi-Pahariya, their real locality²². Which locality the rest of the conodonts have come from seems to be fit for a game of guessing. It has been recently pointed out by Talent and several co-authors²⁵, that seven conodont specimens, illustrated a decade earlier from the Carboniferous of Ladakh²⁶, reappeared in a paper of 1986 (without the co-author) and were said to be from Losar⁸. Gupta points out similarities of this conodont fauna with that of several countries⁸. One can only hope that like the coral specimens from Wales²⁵, resemblance here also does not imply that the conodonts actually come from these countries.

(3) The documentation of some coral specimens of Carboniferous age from "near Surichunla" (ref. 27) and "Bara-lacha Ban" (ref. 28) by Gupta alone, and a later redocumentation from "near Tanze" (ref. 29), this time with Professor M. Kato of Japan, has raised new dimensions to the recycling of specimens. Only Gupta can

say what is the genuine provenance of these corals, but in the light of the above one can rule out an occurrence at Bara Lacha Ban^{20,21}. It also remains to be stated precisely how "near" the alleged occurrence is to Surichun La and Tanze in Ladakh (refs 27, 28). Under these circumstances, even the supposed dates of collection of the samples would be of interest.

Permian

(1) The description of Permian limestone or Sarchu limestone from near Sarchu bridge³⁰ has been found to be untenable by all later workers, not only at the locality mentioned but also in the detailed regional surveys which established the absence of any Permian limestone in the Zaskar, Lahul-Spiti and Kinnaur regions. Triassic and Jurassic rocks were found at Lach Lung La³¹ where Gupta had reported *Eurydesma*. The "Malung Shale" from which Gupta³² reported *Eurydesma cordatum* (Early Permian), is reported to yield typical Upper Triassic fossils³³. Yet Gupta and Waterhouse³⁴ not only wrote another paper reporting *Eurydesma* from the Malung Shale in Ladakh but later followed it up with a report of *Eurydesma cordatum* from the diamictite succession "forming transition between the Po Series below and the Kuling Series above": that is, the Ganmachidam Formation (Upper Carboniferous). This claim is contradictory, for *Eurydesma* has never been reported from anywhere below the level of the Gechang (Kuling) Formation — if Waterhouse and Gupta³⁴ are to be believed, one would end up having a typical index fossil occurring at two distinct and widely separated stratigraphic levels and also have the supposed fusulinid-bearing Sarchu Limestone occupying the level between the two! It is not clear whether it is

Gupta or Waterhouse who is credited with collecting the samples, but the description of the *Eurydesma* locality as "exposed near Ganmachidam (left bank of the Kibijima nala stream) and Losar" (ref. 34) is ridiculous; Ganmachidam is situated in a meadow on the right flank of the Spiti river and not on the "left bank of Kibijima nala" which is a tributary of the Spiti quite some distance away.

(2) Gupta's report of *Popanoceras*³⁵ from "brown to greyish brown sandstone beds exposed in Savita nala near Losar" is not only palaeontologically anomalous but it also is not in conformity with the geological details on the ground. If all his claims are to be believed, this Middle Permian fossil is now found almost at Lower Permian or Upper Carboniferous levels. His locality is defined very imprecisely and a lithocolumn is not given, and yet he seeks to re-identify the stratigraphic horizon in the area as Gechang instead of Ganmachidam! I re-checked this locality, which is very near to the Losar Rest House, only in August–September 1988. "Near Losar in Savita nala", only the lower Middle Carboniferous Po Formation is exposed. High up on the flanks, but far from Losar and nearer to Ganmachidam, one finds lower parts of the Ganmachidam Formation. Could one really ever obtain Middle Permian or Lower Permian index fossils from an Upper Carboniferous sequence? Likewise, the stratigraphic position of the Ganmachidam Formation described by Gupta and Waterhouse³⁶ is full of contradictions and speculations.

Gupta is known to have been to Losar in the Upper Spiti Valley only once, in 1965 with S.P. Jain. There have been confusing reports about visits by J.B. Waterhouse to parts of Spiti and Kinnaur. Only Waterhouse can clarify his exact contributions to

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so many vague and highly anomalous reports from the Himalayas^{34,36-38}, which are unlike his works from other areas. Until 1988 no foreigners were allowed into Spiti, and when they were allowed to do so (as were Talent, Fuchs and a few others) they had to be accompanied by Indians.

Allegations of recycling of fossil specimens by assigning fictitious locality labels, or using foreign materials once housed in foreign museums²⁵ to illustrate 'new dis-

coveries' from the Himalayas, are indeed serious and are being treated as such at Chandigarh, contrary to the report by Jayaraman³⁹. Not only Gupta but also Waterhouse must speak up honestly and boldly, and address the grave charges point by point, as the issues involved can no longer be evaded. □

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Early Devonian ostracodes

S. B. Bhatia

I WOULD like to take this opportunity of placing on record facts concerning the precise locality from which the Early Devonian ostracodes, reported by myself and my colleagues V.J. Gupta and S.P. Jain¹, were collected, and to which Talent referred in his Commentary article. I was a member of the expedition (along with Gupta, Jain and R.C. Kanwar) which visited the Spiti Valley in 1966, and in the last leg (on which Jain was not present) I collected samples from near Kurig, close to the India-Tibet border. But the sample in question (containing ostracodes) was not collected by me. I also do not recollect Gupta collecting samples from that area.

However, several years later, in 1972, when I was about to visit Britain under a British Council Exchange Programme, Gupta handed over to me a small vial containing some washed residue and asked me to have a look at the ostracodes it contained during my stay at the British Museum (Natural History). Gupta stated that the sample was from near Kurig, without being precise, and of Devonian age. My own observations in the field diary, however, were that the rocks around Kurig were of Permo-Carboniferous age. I accepted Gupta's statement in good faith, presuming that I had missed the band containing Devonian ostracodes.

While working at the BM(NH) I was struck by the close similarity and even match, at the specific level, of the material provided by Gupta and the material from the Haragan Formation, Oklahoma, housed in the BM(NH). Accordingly, a slide containing representative ostracodes was sent to Dr Lundin of Arizona State University for his comments, and he too was impressed by the similarity of the Spiti and Haragan faunas.

Be that as it may, as a sequel to the controversy raised by Talent it became imperative for me to recheck the possible match of the Spiti and the Haragan ostracode faunas. Accordingly, I requested Lundin (in a letter of 12 April 1989) for some comparative material from the Haragan. I await that material, but can

quote from Lundin's letter of 2 May 1989:

... I have followed the controversy brought on by Gupta's discoveries and the article by Talent with great interest especially because of the ostracodes you sent me in 1972. I recall that I was especially impressed at the similarity of the fauna you sent me with the Haragan fauna I described in 1968. The species constitution and even the state of preservation of your fauna and the Haragan fauna, as I recall, were remarkably similar. Scientists are skeptics! As well they should be. I regret that your name has been tarnished. ...

The facts mentioned above are being communicated so that the scientific community may know the truth regarding the worth of 'Devonian ostracodes' from the Himalayas in the context of the controversy regarding the authenticity of Gupta's research findings. As stated in the editorial on p.604 of the same issue of *Nature* as Talent's article, palaeontologists should, on this occasion, have been quicker to voice their doubts. True, but then it is better to be late than never.

Finally, I should like to comment on the news story by Jayaraman which later appeared in *Nature*².

Jayaraman's contention that Gupta's colleagues at the Centre of Advanced Study in Geology, Panjab University, Chandigarh, consider Talent's allegations as a "conspiracy to denigrate a top Indian scientist" is not true. I, for one, reacted immediately to the controversy, and in a letter dated 27 April 1989, addressed to our Vice-Chancellor, I suggested that a fact-finding committee should be set up to get to the truth. In fact, as stated in this letter, I am firmly of the opinion that the only way in which the scientific community will be convinced of Gupta's research findings will be for Gupta to invite some Earth scientists to accompany him to a few of the controversial localities in the Himalayas and prove the authenticity and reproducibility of his fossil finds. This is the only way Gupta can redeem his reputation as well as the fair name of our university. □

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The Kinnaur region

Udai K. Bassi

THE Kinnaur region of the Himalayas is the subject of some of the questionable papers discussed by Talent. This inhospitable and rugged terrain was systematically mapped and investigated by myself and my associates during nine expeditions (1978-86), each expedition lasting over a hundred days. Based on my intimate knowledge of Kinnaur and part of Spiti, I make the following observations on publications dealing with this area.

(i) It would be impossible to obtain the Devonian ostracode fauna¹ from Kurig, for in this area only Carboniferous rocks are exposed².

(ii) The report of Late Devonian vertebrate remains³ from the Yulang section states: "Muth Quartzite passes up into a 50 m thick succession of shales with thin bands of quartzite followed by a 200 m thick horizon of siliceous and shaly limestone". Another paper on the same area⁴ states: "The Muth Quartzite in the Yulang River section is conformably overlain by 10 m thick succession of grey limestone forming transitional horizon between the Muth Quartzite below and Lipak Formation above". The lithostratigraphy in the two reports is not only contradictory but factually incorrect — the sequence in the Yulang section is inverted and the Muth is physically overlain by the Silurian Takche Formation. Moreover, Gupta reported the same conodont fauna (*Palmatolepis*, *Polygnathus* and so on), from two limestone horizons separated by 50 m and resting above the Muth Quartzite^{3,4}. The anomalies in lithostratigraphy and fauna levels render these two papers worthless.

(iii) The description of the geology of the Tidong Valley⁵ surprisingly lacks a geological map and contains a borrowed lithocolumn⁶. It also omits details of the pre-Muth sequence, which is best developed in this section⁷. The report of sulcispiriferinids⁸, ignoring the work of Chopra *et al.*⁹ and referring to Gupta and Waterhouse⁵ with a wrong title, is mystifying.

(iv) The paper on the Fammenian ammonoids⁹ post-dates all papers on Khimokul La yet it avoids all references to this work, even Gupta's own earlier papers. The "limestone" or "shale" bands referred to in the Muth Formation in this section are fictitious and do not exist in the field. The paper declares that the repository of the ammonoids is the Geology Department at Panjab University, but no registration numbers are given. Enquiry at the Geology Department revealed that these specimens were never deposited there.

The statement "Noric and Rhaetic suc-

cession is poorly fossiliferous and has not yielded any determinable fossil", attributed to Bassi *et al.* (1980) by Gupta and Waterhouse³, is a misquotation. I have never made such a statement, nor does such a publication exist. The report of Carnian conodonts¹⁰ from the Khimokul La cannot be true as no rocks younger than Lower Triassic exist at this pass on the Indo-Tibet border. Dr O. N. Bhargava and I went to the base of Khimokul La in 1984 to sample the Silurian reef¹¹ and to check Gupta and Erben's report of ammonoids in the area⁹. The problem of the ammonoids apart, Gupta's, Erben's and Waterhouse's names do not figure in the register at the border post; anybody

who wishes to go to Khimokul La has to pass through that post. Further enquiries of local people revealed that neither Gupta nor any foreigner visited this valley, even the fringes of it.

I regret having published three palaeontology papers^{6,12,13} with Gupta. Of these, the report of *Neogondolella regale*¹³ has to be withdrawn because the lithocolumn supplied by me has been changed without my knowledge to increase the thickness of the Triassic by about 30 m, possibly to accommodate the Carnian conodonts¹⁰. □

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Breakdown of trust

Philippe Janvier

THE world community of palaeontologists should be grateful to Dr Talent for his work, Sherlock Holmes style, on 'anomalies' in Himalayan geology. As one of the numerous people who co-authored with Professor Gupta some of the papers in question, I would like to comment upon remarks made both by Talent and in the editorial article in the same issue of *Nature* (p.604).

Talent and several journalists who have written about this case question why so many reputedly good scientists have been taken in by these 'Himalayan' fossils. The process of being caught in such an affair involves both psychological and professional factors. The psychological factor is the trust one has in one's colleagues. I would guess that 90 per cent of the published palaeontological data are based on trust in the field geologist who collected the fossils; when the fossiliferous localities are later revisited, the data are generally confirmed. Given this, most palaeontologists dare not even think it possible that their colleagues may have misled them. Even if they do so, they have a duty to wait for evidence. Presumptions alone are not sufficient.

The first I heard of reservations concerning Professor Gupta's fossils was in 1981, when I had written a paper with him about a most interesting Devonian sarcopterygian fish, supposedly from the Zanskar area of India¹. The same year this fish

turned out to be *Youngolepis*, known elsewhere from Yunnan (China) and North Vietnam.

Talent and colleagues suggest that the presence of this strongly endemic Chinese form in the Himalayas should have been regarded as unacceptable². However, beside the fact that the Chinese example was undescribed when Professor Gupta brought me his specimen, the presumption of endemism cannot be regarded as a scientific argument to reject a finding. Endemism is untestable in palaeontology, and can be approached only by the accumulation of empirical data. Thus, although I had some doubts about the Himalayan origin of the fish, I could only express them, in an additional note, by saying that it was strikingly similar in aspect, colour and matrix to the specimens from Yunnan. Moreover, all the reservations I had heard at that time about Professor Gupta's work sounded like gossip, and I was not prepared to take them seriously until they appeared in print and until Professor Gupta had had the opportunity to defend himself.

French geologists still feel guilty about the Deprat case in the 1920s, when the French field geologist J. Deprat, Head of the Service Géologique de l'Indochine in Hanoi, was accused of having misattributed specimens after finding particular trilobites in Yunnan. Deprat was fired and banned from the Geological Society of France (he later became a novel writer and even wrote a symphony). Some years later, the trilobites in question turned out to be exactly at the right place. The basis for the accusation against Deprat was that the trilobites belonged to the 'Barrandian'

fauna of western Europe, and that some specimens were glued with the same glue as used in the collections of the School of Mines in Paris.

In sum, the reason why Professor Gupta could continue to travel around the world with his 'Himalayan' fossils is that, until the appearance of Talent's article, those who were really aware of problems in Himalayan stratigraphy and tectonics never expressed their doubts in print, and in an unambiguous way. After my experience with the 'Zanskar' fish, I have never published on any fossils I have not collected myself, unless they have been deposited in a museum that can warrant their origin.

Talent describes some of the professional factors behind occurrences such as these. One is the need to publish as many papers as possible in order to become 'famous'. Many professional researchers in palaeontology do so because they are required to do so! Some write virtually the same paper in different languages, others publish accounts of a particular fauna, group by group, sometimes fossil by fossil. In France, researchers are encouraged to do this. The reason is said to be that such an approach makes easier the evaluation of the thoroughness and diligence of the work of a researcher or of a research team. In reality, it facilitates the work of the committees that have to promote researchers: instead of reading the papers, it suffices to count them. I guess that this practice is not unique to France. Clearly it works in India too.

Finally, I would like to stress the difficulty of defining what is a fraud in palaeontology. In the press, the Gupta case has been compared with that of Piltdown. Indeed, in both instances the fossils did not originate in the locality they are supposed to have come from, and the only difference is that the Piltdown site is more accessible than the Himalayan valleys.

But there are all kinds of gradations in wrong-doing and some misdemeanours are far less obvious than others. All of us know of slightly distorted facts or measurements that can be accounted for as simple mistakes, but that have important bearings on the conclusions that are drawn from them. The abundant literature on mass extinctions, for example, is full of such examples, which will never be regarded as true frauds. I am sure that some parts of the literature on molecular biology are flawed with similar cases. These belong to the 'noise' in the history of science. The Gupta case may just be a 'big noise'. □

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Is chaos becoming conversational?

Most accounts of chaotic dynamical systems are based on numerical computations of some kind. But now there is a welcome sign that verbal arguments may make these studies more accessible.

THERE is something waywardly biblical about the ambition to make order out of chaos, but there is nothing wrong with wishing to engage in conversation about the concept of chaos in dynamics even if one happens to be so disadvantaged as not recently to have had access to a Cray II. That thought will be provoked in the minds of many who stumble on a fascinating paper called "Routes to chaotic scattering" in the issue of *Physical Review Letters* for 28 August (63, 919; 1989).

Chaos in dynamics is a simple concept now much popularized, often for the wrong reasons: there are circumstances in which the solution of the equations describing real problems in dynamics depend sensitively, sometimes exquisitely so, on the initial conditions. The result is that identical particles starting from virtually indistinguishable places in phase space (the join of position and velocity) will rapidly drift apart from each other. Those who work in the field and the Cray II seem to have been made for each other, for most of what is known about chaos is numerical.

What S. Bleher, E. Ott and C. Grebogi from the University of Maryland have done in their article will encourage those with different inclinations. Their arguments are in the main verbal, and almost analytical.

Their problem, which evidently should have been taken up much earlier, is simply that of the scattering of classical particles (otherwise 'billiard balls') by a potential surface, a region of space in which a particle has extra potential energy and from which it is therefore repelled. That such a system should be capable of chaotic behaviour is easily appreciated: even in the over-simple representation of a scattering centre by a billiard ball, the outcome of a scattering event is too often an inconveniently sensitive function of the impact parameter (the offset of the direction of travel of the particle and the parallel line through the centre of the target), as billiards players know all too well.

For kinds of scattering centres other than rigid hard spheres, the behaviour of impinging particles can be more complicated. For example, in nuclear scattering (still classical), there is supposed to be a region of space in which the potential energy is spherically symmetrical about some point (the nucleus). The potential increases steadily as the scattering centre is approached and will then be a maxi-

mum, say E_m . Rutherford was the first to calculate the scattering angle as a function of the impact parameter.

In this simple case, chaos does not arise. Indeed, if the energy, E , of the impinging particles is greater than E_m , it is easy enough to guess what the graph of the scattering angle against the impact parameter will be. When the impact parameter is very large and, alternatively, zero, the scattering angle will be zero: particles whose paths are a long way from the scattering centre will not be deflected, whereas those aimed directly at the centre will also go straight through, but there will be some intermediate impact parameter for which the scattering is a maximum, but the maximum angle must be less than $\pi/2$. The case when E is less than E_m is more complicated, for particles aimed directly at the scattering centre bounce right back, but chaos does not arise.

But what if the scattering centre is not a simple potential-energy peak, but a group of several of them? This is the problem that Bleher and his colleagues tackle, for simplicity in two dimensions. As a numerical example, they describe what happens when the potential energy is a simple function of the coordinates of the form $x^2y^2\exp[-(x^2 + y^2)]$, which is really a system of four equal peaks in the potential-energy surface. When E is greater than E_m , again there is no difficulty; the scattering angle is a more complicated function of the impact parameter, but still smooth. But if the energy is less than the maximum, the system lapses into chaos. What happens is that particles are trapped within the system, bouncing repeatedly between the energy maxima before eventually escaping in a direction that is exquisitely sensitive to the manner in which they were originally captured.

What can be said in words about such a complicated problem? A great deal, it seems. The authors take the general case of an array of three scattering centres whose energy maxima are E_{m1} , E_{m2} and E_{m3} , arranged in order of increasing size. Two cases arise, the authors say, depending on the geometrical relationship of the scattering centres. Either the weakest of the three lies outside the circle whose diameter is the line joining the other two, or it lies inside that circle. The essence of the distinction is that, in the first case, the angle at the apex of the triangle joining the three scattering centres must be less than $\pi/2$, but otherwise is greater than that.

The problem is to predict when and how particles impinging on this system will behave chaotically.

Chaos plainly cannot arise unless E is less than E_{m2} , for then there is no way in which the particles can be trapped into orbits bouncing between a pair of scattering centres. And, in this case, because the maximum scattering angle at the lowest peak must be less than $\pi/2$, a particle scattered from say E_{m2} towards E_{m1} cannot be scattered back towards E_{m3} . But once the energy of the incident particles falls below E_{m1} , an infinite number of trapped orbits is accessible, simply because larger angles of deflection are immediately possible. There is an abrupt transition from order to chaos.

The second case, where the scattering centre with the smallest energy lies within the circle, is more complicated. If E is greater than E_{m1} but less than E_{m2} , everything hangs on whether E_{m1} is large enough to deflect a particle arriving from one of the other scattering centres towards the third. If so, there will again be an immediate transition to chaos, for an infinite number of nearly periodic bouncing orbits will be accessible to particles. If, on the other hand, E_{m1} is insufficiently large for that, there will still be trajectories in which a particle first scattered by E_{m1} will be trapped for less or more time between the other two centres. The number of these orbits will multiply rapidly as E is reduced below E_{m2} , but in a manner exactly like the appearance of chaos in more familiar chaotic systems, by the process called bifurcation (equivalently, 'period-doubling') in which extra allowable orbits make their appearance as some parameter is varied.

The result is simple enough, but plainly applicable to much more complicated arrays of scatterers. To those working in the field of chaos, much of the interest will lie in the authors' demonstration that chaos can arise in the same dynamical system in two distinct ways — either abruptly, or by the more familiar bifurcation route. For what it is worth, their calculation is that the fractal dimension of the number of chaotic orbits varies as they expect with the difference between E and the energy of the scattering peak. But, to others, it will be enlivening that the authors have been able to make their intricate argument almost verbal. Those without an allocation of time on a Cray II will be pleased.

John Maddox

Binding of infected red cells

Louis H. Miller

NEARLY a century ago, Bignami and Bastianelli noted that red cells infected with the malaria parasite, *Plasmodium falciparum*, disappear from the peripheral blood. It has since emerged that they are sequestered along the smaller blood vessels, by which means the parasites are protected from the lethal immune activity of the spleen. That same process is responsible for many of the complications of

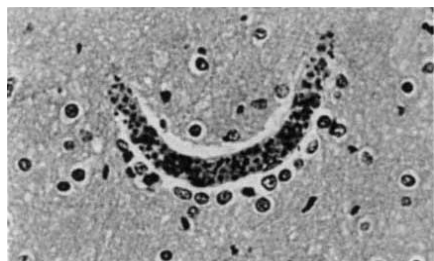


FIG. 1 A cerebral capillary from a cerebral malaria patient. The capillary lumen is obliterated with infected red cells.

severe malaria and for much of its mortality. Thus the placentae of fetuses carried by infected mothers are filled with parasitized red cells, which may contribute to low neonatal weight and to high maternal mortality (especially in a first pregnancy), whereas infected red cells bound to vessels in the brain (Fig. 1) may account for the high death rate in cerebral malaria, which is well in excess of 20 per cent.

These facts give sufficient reason for the exploration of the molecular basis of the cytoadherence of infected red cells, now well under way. Berendt *et al.*, reporting on page 57 of this issue¹, have identified intercellular adhesion molecule-1 (ICAM-1) as a third cell-surface receptor for infected red cells; furthermore, the authors have demonstrated that different parasite clones stick to different receptors — cytoadherence is heterogeneous. In due course, that could lead to novel therapies for some of the complications of malaria.

The molecular mechanism by which infected red cells, exploiting knob-like protrusions on their membranes (Fig. 2), bind to the surfaces of venules can be studied because of continuous-culture methods for *P. falciparum*² and *in vitro* techniques for the study of cytoadherence^{3,4}. Most work in the field has so far relied on human umbilical endothelial cells³ and a melanoma tumour line⁴, but the expression of specific membrane proteins in transfected cells, as with the expression of ICAM-1 in COS cells¹, which transiently express genes at high level, is a more direct route to understanding the details of specific binding.

As yet, there is no direct proof as to which molecules on the red-cell surface mediate attachment to endothelium —

one candidate is immunoprecipitated by strain-specific sera⁵ that also block cytoadherence in a strain-specific manner⁶. By contrast, the new report brings to three the number of identified host proteins to which infected red cells bind. One is the soluble protein thrombospondin⁷, another is CD36 (see refs 8,9) which, like ICAM-1, is a surface membrane protein expressed on specific cells. Binding has been demonstrated either to isolated protein or to cells transfected with the appropriate gene.

Several questions are provoked by this multiplicity of host binding proteins. Are parasitized red cells generally sticky, binding to several proteins? If so, do these proteins have a common group at which binding takes place? Otherwise, what is the physiological significance of the parasitized red cell's capacity to bind to three different host proteins? Which of these, if any, are involved in the sequestration of parasitized red cells to endothelium in uncomplicated malaria, to cerebral vessels in cerebral malaria and to the placenta? Binding to various melanoma-cell lines correlates with the expression of CD36, but that does not define the mechanism of binding in human beings.

The study by Berendt and colleagues¹ marks a start on the unravelling of that problem. Different *P. falciparum* clones, ITO4 and FCR3, are shown to bind to different molecules. But the observation that ITO4 clones bind to COS cells separately transfected with ICAM-1 and CD36, and that FCR3 clones bind only to the latter, in itself suggests that there are least

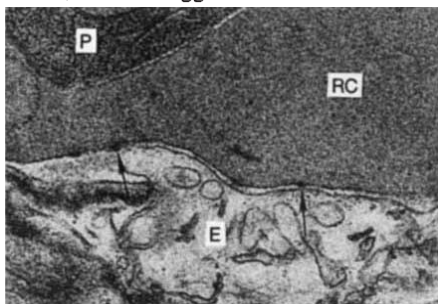


FIG. 2 A cerebral capillary with *P. falciparum* (P)-infected red cell (RC) adherent to the endothelial cell (E) by knobs (arrows). (From ref. 14.)

two distinct binding mechanisms and that red cells, as modified by parasites, do not recognize determinants common to the two host receptors. That is consistent with the absence of homologous regions in the amino-acid sequences of ICAM-1 and CD36. The binding specificity of parasites from patients with cerebral malaria will be awaited with great interest.

The cytoadherence molecules on parasitized red cells would be another part of the story, but perhaps only a part. In

animal models, *P. falciparum* sequesters not in cerebral vessels, but in venules elsewhere in the body¹⁰. Cerebral malaria caused by the rodent parasite *P. berghei* is associated with macrophages and parasitized red cells in the cerebral vessels, and tumour necrosis factor (TNF) has been shown, by antibody blocking experiments, to have a role in the disease¹¹. Although macrophages are not found in the cerebral vessels of people with cerebral malaria, seriously ill patients have markedly high levels of TNF (ref. 12).

Given that cultured human endothelial cells express ICAM-1 on the addition of TNF, it is possible that TNF (and other cytokines) may enhance the expression of molecules on the cerebral endothelium to which malarial red cells bind, with the consequence that cerebral vessels are obstructed. That implies that there is now a need to define the putative receptors on human cerebral endothelium when there is, or is not, cerebral sequestration. The outcome might be anti-receptor therapy to reduce the mortality from this dread complication of infection with *P. falciparum*.

The biology of the diversity of mechanisms of cytoadherence is also important. The evolution of cytoadherence, it must be recalled, has been driven not so that parasites can block cerebral blood vessels, but so that they may avoid circulation through the spleen. So why are there several parallel mechanisms? It is a little like the diversity of mechanisms for invasion by parasites, where the pathway used is determined by the characteristics of the red cell and of the parasite clone; *P. falciparum* merozoites may invade red cells by pathways that are dependent on, or independent of, sialic acid, for example¹³. The diversity of receptors both for invasion and cytoadherence may enhance the success of each, thus increasing the chances of survival of parasites living in the blood under continuous immune attack. □

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Losing symmetry by design

Kenneth D. M. Harris and Mark D. Hollingsworth

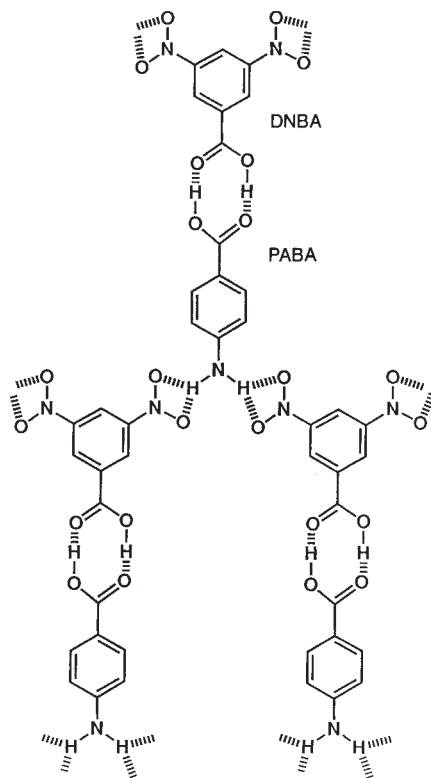
CRYSTALLINE organic solids are becoming increasingly important for their optical, electrical and magnetic properties. In general, these depend not only on the properties of the constituent molecules, but also, sometimes critically, on the spatial arrangement of the molecules in the crystal. Progress in the design of molecular crystals with carefully tailored solid-state properties clearly relies upon an improved understanding of the factors that control crystal packing. Many successful, albeit largely empirical, strategies have been devised for engineering molecules to crystallize in controlled and predictable arrangements¹. New advances in the 'crystal engineering' of hydrogen-bonded structures, discussed by M. C. Etter (University of Minnesota) at a recent conference*, are now enabling the design of materials with important non-linear optical properties.

Hydrogen bonds are ubiquitous in organic crystals and, because they are often stronger than other intermolecular forces, they frequently control the molecular packing arrangement. By surveying the extensive crystallographic data in the Cambridge Structural Database, Etter and co-workers have developed a systematic rationalization of hydrogen bonding patterns. They used graph theory to classify recurring patterns in a large number of hydrogen-bonded structures, allowing them to establish a set of empirical rules to categorize the features of hydrogen-bonded linkages formed by various specific types of functional groups. As a result, it is now possible to predict, with a good chance of success, the probable mode of crystal packing of molecules designed to have strategically arranged hydrogen-bond donor and acceptor groups.

A principal goal for Etter's group is the design of frequency-doubling crystals. Because these crystals convert light of one frequency to light with twice the frequency, they can greatly increase the frequency range of lasers, and have numerous potential applications in the emerging field of optoelectronics. The parameter that characterizes the usefulness of an individual molecule for frequency doubling — otherwise known as second-harmonic generation — is its second-order hyperpolarizability tensor, denoted β .

Molecular features such as polarizable π -electrons, donor-acceptor conjugate pairs or long conjugated chains generally give rise to a large value of β . In a crystalline material, however, second-harmonic

generation also depends on the spatial arrangement of the molecules. For example, a crystal cannot exhibit second-harmonic generation if its structure has a centre of symmetry, even if the constituent molecules have a large β . Further crystallographic criteria have been identified by Zyss and Oudar², and other factors, such as phase-matchability and



The co-crystal of *p*-aminobenzoic acid (PABA) and 3,5-dinitrobenzoic acid (DNBA) comprises a stacking of layers of the type shown. Each layer contains an extensively hydrogen-bonded, non-centrosymmetric arrangement of the molecules. (The hydrogen bonds are indicated by dashed lines.)

absorptivity, also determine the ultimate usefulness of a material for frequency doubling.

Unfortunately, although in some ways predictably, many molecules that have a large value of β tend to crystallize spontaneously in centrosymmetric structures: hence the interest of Etter and others in developing crystal engineering strategies to induce such molecules to adopt acentric structures. Etter points out that a single hydrogen bond is intrinsically non-centrosymmetric so that non-centrosymmetric arrays can be constructed from judiciously designed molecular building blocks joined by strategically positioned hydrogen bonds. In designing materials suitable for second-harmonic generation, the

approach is to choose a molecule with features promoting a large value of β , and to arrange hydrogen-bond donors and acceptors in positions that promote non-centrosymmetric aggregation.

One notable success of this strategy, reported by Etter and Frankenbach earlier this year³, is the co-crystal of *p*-aminobenzoic acid and 3,5-dinitrobenzoic acid, which has the packing depicted in the figure. As predicted, the molecules form a non-centrosymmetric, two-dimensional sheet which is held together by an extensive network of hydrogen bonds. The stacking of these acentric, polar layers is harder to predict: the individual layers are, in fact, buckled so that they stack on top of one another with translational symmetry. The polarity of the layer is, therefore, transferred to the crystal as a whole and the material is capable of second-harmonic generation. (Unfortunately, the molecular dipole and a crystallographic 2-fold axis coincide, resulting in a loss of phase-matchability, and hence of performance.)

Several other approaches for inducing molecules into non-centrosymmetric environments are being explored. For example, thiourea inclusion compounds containing certain organometallic complexes — such as $(\eta^5\text{-benzene})\text{Cr}(\text{CO})_3$ — can give pronounced second-harmonic generation⁴. The pure (native) crystalline phases of these organometallic materials are centrosymmetric and therefore incapable of second-harmonic generation, but when included within the linear tunnels of their thiourea inclusion compounds, they adopt a non-centrosymmetric head-to-tail dipolar alignment. The inclusion of an organic molecule (*p*-nitroaniline) within an acentric aluminophosphate molecular sieve (ALPO-5) has also been used to produce second-harmonic generation⁵.

Another important study⁶ has shown that 'guest' molecules occluded within centrosymmetric 'host' crystals can be selectively incorporated into certain symmetry related sectors of the crystal but not into others. Even extremely small amounts (0.01 per cent weight) of guest can lead to a sufficient lowering of the symmetry of the crystal for significant second-harmonic generation to be possible. □

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* 9th International Conference on the Chemistry of the Organic Solid State, Como, Italy, 2-7 July 1989.

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Watching neurons discriminate

M. J. Morgan

WHAT is the nature of the perceptual decision process in the brain when we recognize an object, distinguishing it from others — a cat from all dogs, for example? How many neurons are involved? How many neural stages are there between stimulus and response? These questions are tackled in a technical *tour de force* by Newsome, Britten and Movshon on page 52 of this issue¹. The authors describe recordings from single neurons in monkey cortex while the whole animal makes a perceptual decision. Their conclusion is that perceptual decisions may be correlated with the activity of a small number of neurons, perhaps as few as one.

Expectations in this field are sharply polarized. In one theory of sensory processing², sometimes known as the 'grandmother cell' hypothesis, perceptual stimuli are categorized by means of a hierarchy of neural filtering mechanisms terminating in the activity of a set of neurons, each of which is associated with a particular object. Crudely, we recognize a cat when the firing rate is higher in the 'cat' neuron than in competitors such as 'dog' or 'rabbit'. The 'cat' neuron is the site of convergence of combinations of perceived attributes that reliably distinguish cats from other animals.

Those who believe perception to be a deeply complicated process find the grandmother-cell hypothesis too simple to be true. That is why there is no shortage of suggestions that perceptual classification depends on distributed activity among large populations of neurons, seamlessly connected with another large population of neurons by which our responses are determined³. In this model, there is a "distributed representation" of external stimuli; one would look in vain for single neurons whose activity is correlated with perceptual decisions.

Previous studies in this field have dealt with these issues only indirectly, by comparing the measured performance of single neurons and of whole animals, but under different conditions and not simultaneously. Thus the capacity of an individual neuron to distinguish between two stimuli can be measured by the difference between the number of spikes evoked by one stimulus and another; if the difference is statistically significant against the background noise of random neuronal firing, we may conclude that the neuron can distinguish between the stimuli. Analogously, but separately, the ability of a conscious monkey or of a human observer to distinguish between two stimuli can be measured by the magnitude of the difference between them that can be detected with statistical reliability.

The statistical criteria have the same meaning in the two approaches, and provide a direct comparison of the capacity of single neurons and of conscious observers to make perceptual decisions. Using this logic, for example, Parker and Hawken have shown⁴ that the capacity of single neurons in monkey visual cortex to distinguish different positions of a line within their receptive field is similar to the ability of human beings and monkeys to report the direction of the displacement of a line in a binary decision task.

Newsome *et al.* break new ground by recording neuronal and whole-animal performance simultaneously. They have recorded from neurons in a monkey cortex while the animal is attempting a perceptual decision — that of identifying the direction of motion across a computer screen of a set of drifting dots embedded in a set of randomly moving dots (to complicate the task). Neurons were selected from the MT area of the cortex (otherwise V5), strongly implicated in motion processing in humans^{5,6}. The first step is to determine for each neuron (60 in two monkeys) the 'preferred direction' of movement, for that in which the neuronal response is greatest. The monkey is then given the task of distinguishing, for each neuron, between motion in the preferred and the opposite direction. Its decision is inferred from the direction in which its eyes move.

The capacity of single neurons to discriminate turns out, in many of the 60 cases, to be as good as the whole animal's; some were even better. One way of thinking about this result is to imagine a contest between two human observers presented with the same task, one of whom is able to examine the spike trains of the neuron and the other only to look at the moving stimulus. Since the acuity of visual discrimination in human beings and monkeys is usually very similar, the results of Newsome *et al.* imply that the two observers would be correct equally often. The outcome of the contest would hang on the discriminatory capacity of the selected neuron whose spike train is accessible. The contestant with access only to the spike train might even win.

Two conceptually difficult questions are provided by their experiments. The first is the problem of identifying the level of processing at which a neuron is active. It is not immediately clear from the result reported so far whether the MT units are closest to the sensory input stage, the perceptual decision stage or the output stage. The standard engineering model of neural processing sees it as a cascade of stages beginning in the retina and ending with some overt behaviour. At each stage,

noise can arise that degrades performance and which is uncorrelated with noise entering at other stages. It follows that if a neuron is found that has a better performance than the whole animal, it is nearer to the sensory-input stage than the final decision process.

Thus the important aspect of the experiment by Newsome *et al.* is not that neurons were found that correlated with the stimulus — this is trivial — but that they were no better than the whole animal. The fact that some were a bit better is an objection to treating them as decision units, as the authors note. In the remaining units, which were no better than the whole animal, the clearest evidence for a real relation between their activity and the decision process would be a trial-to-trial correlation between neural activity and the monkey's response when the influence of the stimulus *per se* was removed by methods of partial correlation. We could then be sure that the MT unit was on the watershed between stimulus and response, rather than too far towards the stimulus side.

It is possible, of course, that the MT units are merely output devices. That they might directly control eye movements (the monkey's signal of its decision) is ruled out by the time-lag between their activity and the movement of the eyes. The question of whether they betoken an intention is metaphysical.

The second deep issue provoked by the experiments of Newsome *et al.* is that of neuronal selection. Only a small population of neurons may be capable of solving the task required of the monkey, but how are just those neurons selected by control of the decision process from a population of many millions? Presumably, monkeys do not carry a perceptual representation of their own nervous systems, so that selection must be a consequence of learning from external stimuli. The problem of selection would be made conspicuous and susceptible to study if it proved that the link between neuronal activity and perceptual decision could be broken by changing the nature of the stimulus in trivial ways and seeing whether the cells and the monkey still agree. For example, the size or colour of the moving dots might be changed. If such alteration of the stimulus were found to make the performance of neurons collapse, without affecting the animal, we could infer that the decision process had switched to another neuron or set of neurons — but that would return us to the selection problem. How was that neuron or set of neurons selected?

The question of how a neuronal process is reinforced so that it comes to depend on a particular stimulus is familiar in studies of learning, but how the mechanism functions at the level of the neuron is still a matter of speculation. Early in the development of learning theory, such questions

occupied the attention of people such as Lashley in the United States, Konorski in Poland and, notably, Hebb in Canada, but in recent years, learning theory has become more distant from neurophysiology. This is a great pity, but the experiments of Newsome *et al.* and the recent investigations of the neural basis of conditioning in the cerebellum⁷ may rekindle interest in the fundamental questions of the neuronal influences of learning.

One feature of the experiment may have contributed greatly to its success. The monkey indicated its response by moving its eyes in the direction of stimulus motion. This is a very natural or, in the terminology of learning theory, 'prepared' response, which may have eliminated the need for a complex decision process between stimulus and response. If we ask what the nature of the decision process might be between MT and eye movement, the answer could be "none". Workers on visual perception and on eye-movement control mechanisms can be

likened to two gangs of workmen tunnelling through the nervous system from opposite ends. They will meet soon and, on the evidence so far, they will do so without having to remove obstacles labelled 'perceiving' or 'decision process'. Such simplicity may be peculiar to eye-movement control, or it may be because this is the only stimulus-response system in primates that we understand in such detail. □

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PALAEOCLIMATE

The desert shall rejoice

Wayne M. Wendland

MOJAVE Desert, which lies between Las Vegas and Los Angeles, and includes Death Valley, is the most arid region in North America. But sediments show that more than 8,000 years ago, this area was a massive lake. Detailed studies of the sediments, reported by Enzel *et al.* elsewhere in this issue (*Nature* **341**, 44–47; 1989), show that the area supported lakes for prolonged periods about 3,600 and 400 years ago, as well. The obvious inference is that the changes indicate significant variations in climate during these periods.

To translate the kind of environmental information used by Enzel *et al.* into reconstructions of the palaeoclimate, it is necessary to find modern analogues that can be related to known weather patterns. Geological, geomorphic, archaeological and botanical evidence have all been used in this way. Although the conclusions from one such exercise can be equivocal, confidence clearly must grow in a climatic reconstruction if independent types of evidence indicate the same conclusion.

How reliable are such reconstructions? Apparently, they are quite good. The location of the main features of atmospheric circulation (the sub-tropical highs, sub-polar lows, jet streams and so on) have remained fairly constant throughout the Holocene (the past 10,000 years), as have their mean intensities and variance. This is not so surprising, as the main components forcing climate — Earth's orbit and the topography and distribution of land masses — have not changed significantly over this period. So modern

analogues are reliable guides to the earlier climate, and together these indicate the variability of the Holocene weather.

The CLIMAP and COHMAP projects are prime examples of concerted efforts of research of this type. The participants in

6 °C colder. Interestingly, all central oceans exhibited no change at about 20° S latitude.

In a somewhat different approach, a component of the COHMAP project involves reconstructing pollen assemblages for many locations based on general circulation characteristics for various times in the past 18,000 years (see T. Webb, P. J. Bartlein & J. E. Kutzbach in *North America and adjacent oceans during the last glaciation* (eds W. F. Ruddiman & H. E. Wright Jr) 447–462 (Geol. Soc. Am., Boulder, 1987)). The prehistoric general circulation is generated by a numerical circulation model whose boundary conditions are known sea levels and glacial extent for those times. The reconstructed pollen assemblages from the model match the extant record to a remarkable degree, thus verifying the method.

Enzel *et al.* link the existence of the perennial lakes they identify in the Mojave Desert to circulation patterns over the central and eastern Pacific Ocean. The modern analogues they use are eight episodes of increased rainfall since 1894 that led to the formation of ephemeral lakes, lasting 2–18 months, in the terminal basins of the Mojave River. The full climate record, including temperature, pressure, wind and humidity observations for the surface and upper air extends back only four decades, but the authors assume that it represents adequately the variance of the main features of the circulation pattern.

The climate of southern California and the San Bernadino Mountains is essentially mediterranean — warm and dry in summer, wettest in the winter. This is related to the seasonal migration of the polar jet stream, associated surface fronts off the Pacific and the Pacific sub-tropical anticyclone. Times of increased rainfall tend to be linked to an anomalous displacement to the south of these circulation features.

The mean sea-level pressure on the Californian coast tended to be strongly depressed during the wet episodes,

and probably forced on-shore flow and moisture over southern California into Mojave. The inference is that similar sea-level pressure patterns persisted during the periods when perennial lakes were found in the Mojave. This is a significant addition to the data for this region and period, and the way it fits into the global pattern of inferred pressures will be fascinating to see. □

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IMAGE
UNAVAILABLE
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REASONS

Aridity in Death Valley, Mojave Desert — a cause for caution.

CLIMAP (*Science* **191**, 1131–1144; 1976) gathered extant data on the concentration of plankton from deep-sea cores of the world's oceans to reconstruct sea-surface temperatures representative of Augusts 18,000 years ago, comparing variations with a modern relationship between plankton and sea-surface temperature. They found that sea-surface temperatures in the high latitude Atlantic and Pacific Oceans were depressed by as much as 4 °C relative to today. Along the west coast of South America and in the central Pacific near the equator, temperatures were up to

Thrombogenesis linked to atherogenesis at last?

James Scott

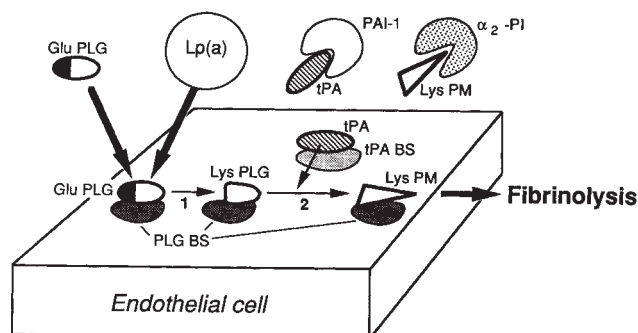
ARTERIAL thrombosis, the cause of heart attacks and strokes, is usually precipitated by rupture of an atherosclerotic plaque in a blood vessel. Despite this devastating and frequently fatal association, there has been no evidence linking thrombosis to the earlier process of plaque formation. A flurry of activity reported in several papers¹⁻⁸ and at two recent conferences* now shows that lipoprotein(a), a variant of low-density lipoprotein (LDL), the cholesterol-carrying augury of atherosclerosis, promotes coagulation and is an independent inherited risk factor for atherosclerosis.

Lipoprotein(a) (Lp(a)) consists of one (or two) molecules of apolipoprotein(a) (apo(a)), linked by a disulphide bridge to apo B-100 (refs 9, 10). It has several size isoforms, the amount of these in the circulation being strongly genetically determined^{11,12} — high levels are a significant risk factor for atherosclerosis. Apo(a) is similar to plasminogen, from which the enzyme plasmin, that dissolves fibrin blood clots, is released by tissue plasminogen activator (tPA). Indeed, the human apo(a) and plasminogen genes are close together on chromosome 6 (ref. 13). The apo(a) molecule has 37 or more repeats of plasminogen 'kringle VI', the 36th of which contains the cysteine residue that forms the disulphide bridge to apo B-100. Both proteins are produced by the liver, and are probably linked by disulphide isomerase in the rough endoplasmic reticulum (ER). Apo B-100 is also a nucleus for lipoprotein assembly in the ER and Golgi apparatus of liver cells¹⁴.

Various data¹⁵ argue against Lp(a) having a serine protease function: rather, three recent studies suggest that it is a physiological inhibitor of plasminogen activation and so prevents unopposed plasmin generation¹⁻³ (see figure). Plasminogen-binding sites are widely distributed on peripheral blood cells and on vascular endothelial cells, and cultured endothelial cells also secrete and bind tPA. The proximity of the binding sites for plasminogen and tPA accelerates tPA-mediated plasmin generation, and surface binding shields plasmin and tPA from their inhibitors.

Lp(a) has now been shown¹⁻³ to compete with plasminogen for the plasminogen binding site with an equivalent affinity and capacity, but to have no effect on the fluid-phase activity of plasmin. The kringle domain is needed for plasminogen and

Lp(a) binding. It has been estimated that at plasma concentrations of 30 mg dl⁻¹, Lp(a) reduces cellular plasminogen binding by 20 per cent, thereby suppressing endothelial cell fibrinolysis and producing a procoagulant state. In addition, there is a striking accumulation of Lp(a) on the endothelium of atherosclerotic coronary arteries, but not in normal blood vessels². Plasminogen is readily detectable in normal vessels, but not in atherosclerotic vessels. These observations provide the most direct link so far between thrombo-



The endothelial cell fibrinolytic system. N-terminal Glu-plasminogen, Glu PLG; N-terminal Lys-plasminogen, Lys PLG; Lys-plasmin, Lys PM; plasminogen-binding site, PLG BS; tPA-binding site, tPA BS; α_2 -plasmin inhibitor, α_2 -PI; plasminogen activator inhibitor-1, PAI-1. 1, Conversion of Glu PLG to Lys PLG by surface-localized plasmin-like diisopropylfluorophosphate-sensitive serine protease. 2, Activation of plasminogen by tPA.

genesis and progressive atherosclerosis.

Further support for the aggressive role of Lp(a) in atherosclerosis comes from studies of plasma Lp(a) concentration and size isoforms in familial hypercholesterolaemia (FH), a condition caused by defects of the LDL receptor gene^{4,5}. Individuals with FH show a threefold increase in plasma Lp(a) irrespective of their isoform phenotype, and those with the highest Lp(a) are at greatest risk of heart disease. The elevation of plasma Lp(a) is comparable to the two- to threefold elevation in LDL cholesterol seen in FH, suggesting that defects of the LDL receptor could cause delayed catabolism of Lp(a) as well as of LDL. There is controversy about whether Lp(a) binds to the LDL receptor on cultured cells, as drugs that lower plasma LDL by increasing the number of LDL receptors do not affect plasma Lp(a) concentration, suggesting that Lp(a) is not cleared by the LDL receptor⁴. Perhaps the elevated Lp(a) in FH is due to increased synthesis, as seems to occur for LDL¹⁶. More probably, the excessive burden of LDL in the circulation in FH interferes with the normal clearance of Lp(a) by one or several possible clearance routes — the hepatic asialoglyco-

protein receptor; the macrophage scavenger receptor, which converts the macrophage to an atherogenic foam cell; or the plasminogen-binding sites on circulating monocytes¹⁻³.

Two of the new reports^{4,5} have significant implications for the management of FH and other forms of hypercholesterolaemia. It is important to ascertain whether individuals with hypercholesterolaemia and heart disease have the Lp(a) phenotypes normally associated with high circulating concentrations and whether reduction of both Lp(a) and LDL is needed to prevent atherosclerosis. That the latter is the case is suggested by earlier studies showing that people with normal cholesterol but with Lp(a) higher than 30 mg dl⁻¹ have double the risk of heart disease¹⁰.

The concentration of Lp(a) in the circulation varies from almost undetectable to more than 100 mg dl⁻¹ (ref. 10). The size of apo(a) ranges between 450,000 and 750,000 daltons, and in humans there is an inverse relationship between the size of apo(a) and the level of Lp(a)^{11,12}. The variation in size of Lp(a) could result from variation in the number of kringle repeats⁹. Three recent studies⁶⁻⁸ on Lp(a) in monkeys have helped to clarify these issues. The size of plasma apo(a) in the circulation correlates closely with apo(a) messenger RNA size — size isoforms are inherited as independent alleles, suggesting that in monkeys variation in kringle number encoded by the gene determines Lp(a) size. Lp(a) concentrations and phenotypes co-segregate, and more than one concentration can be associated with a single size isoform. The amount of Lp(a) in plasma corresponds to apo(a) mRNA concentrations, suggesting that the Lp(a) level is established by transcription rate. Thus apo(a) size and concentration alleles are found at the apo(a) structural locus and seem to depend on different properties of the gene. Discrepancies between mRNA and plasma levels suggest that post-transcriptional mechanisms are also operative. For example, a null allele produces significant levels of mRNA, but only traces of Lp(a) are detectable in the circulation.

Lp(a) plasma concentrations in humans are also influenced by metabolic factors. Thus Lp(a) is increased in diabetes mellitus and nephrotic syndrome; decreased by dietary fish oil and by combined nicotinic acid and neomycin treatment, but little affected by diet^{6,10} (I. Karadi, Semmelweis

* International Atherosclerosis Congress, Vienna, 20–22 April 1989; From Phenotype to Gene in Common Disorders, Oslo, 18–20 May 1989.

University; K. Berg, University of Oslo; G. Dahlen, University of Umeå). Most of these situations influence plasma apo B levels in the same direction, and act in part by affecting lipoprotein biosynthesis in the liver^{8,14,17-21}. Taken together, the evidence suggests that the metabolic regulation of Lp(a) production is linked to that of apo B-100 once they have been covalently bonded in the endoplasmic reticulum. If this is so, how can the lack of correlation between LDL and apo B and Lp(a) levels be explained¹⁰? That LDL and Lp(a) are cleared by different routes and that excess apo B-100 is always available in the ER for linking with apo(a) suggests an answer. It

is also possible that Lp(a) metabolism follows a different intracellular pathway from that of other liver lipoproteins. More probably, the fate of apo B-100-containing lipoproteins in the secretory pathways will determine the fate of Lp(a). The production of apo B-100 containing lipoprotein particles is regulated by post-translational mechanisms — partly by sorting between intracellular protein degradation and secretion¹⁴. □

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LOW-MASS STARS

Protostars in from the cold

A. P. Whitworth

UNTIL recently, little was known about the origin of low-mass stars (those whose mass is no more than 2 solar masses). With a few notable exceptions, astronomers had tended to concentrate on high-mass stars, seduced by the spectacular emission nebulaosity that follows the birth of a high-mass star, but which reveals little about its conception or gestation. Following a recent meeting*, it is clear that this bias has been more than redressed. The observational picture of how low-mass stars form is now extremely detailed and is rapidly being translated into a much better theoretical understanding of the processes involved. By contrast, high-mass stars still remain something of a mystery.

There are two reasons for this turn-about. The first is the opening up of new windows in the electromagnetic spectrum. Throughout the late 1960s and well into the 1970s, optical and radio astronomy held sway. Observations were focused on and around H II regions (bright emission nebulae surrounding young hot stars). Although this inevitably selected sites of high-mass star formation, it was widely presumed that low-mass stars formed in the same places. With the rapid development of infrared and sub-millimetre observing techniques in the last decade, it has become possible to study the more

quiescent regions of star-forming cloud complexes. The result has been to bring into focus regions like the Taurus–Auriga cloud complex where only low-mass stars are forming.

The second reason has more to do with theoretical necessity. It seems inescapable that the circumstances producing the globular clusters in the halo of our Galaxy differ fundamentally from those responsible for the OB associations (small, unbound groups of young, massive stars) in the galactic disk. It would be extraordinary if all star formation followed a single pattern and that variations were due solely to statistical fluctuations. Yet, until recently, this is essentially what astrophysicists were obliged to assume, there being no hint of which circumstances exerted which influence on the course of star formation. To have assumed that the efficiency of star formation or the initial mass function depended in some systematic way on epoch or environment would have been blind speculation.

It is now known that low-mass stars form more or less continuously, but very slowly, throughout the volume of cold, quiescent, dark clouds like Taurus–Auriga as these clouds cruise round the galactic disk (J. Bally, AT&T Bell Laboratories). High-mass stars tend to form only in the most dense parts of larger, hotter and more turbulent clouds; their

formation is concentrated in the spiral arm regions of the galactic disk and seems to be triggered by compression, presumably in a galactic shock.

Quiescent regions of low-mass star formation tend to possess an ordered large-scale magnetic field structure (K. Strom, University of Massachusetts), as revealed by polarization and Zeeman measurements. Because the rotation axes of young stellar objects in these regions are well aligned both with one another and with this background field, we conclude that the magnetic field plays a dominant role in the formation of low-mass stars.

The most plausible interpretation of this observation is that, although the condensation of low-mass stars is driven by their self-gravity, it is tightly controlled by the entrained magnetic field (S. Lizano, University of Florence). The magnetic field slows down the early stages of condensation because the neutral particles (which constitute most of the matter) experience a friction force as they slip past the charged particles (which are tied to the magnetic field lines by the Lorentz force). The resulting quasistatic condensation takes 10^6 – 10^7 years and results in the formation of dense, centrally concentrated cores with properties similar to those observed in Taurus — having protostellar masses about that of the Sun and radii about 10^{15} m.

The entrained magnetic field also exerts the torque that reduces the angular momentum of a condensing protostar, thereby enabling it to reach stellar densities without spinning so fast that it breaks up. This has the effect of aligning the spin axis of a condensing protostar with the background magnetic field and hence with the spin axes of other protostars in distant parts of the cloud, as described by Strom.

The spin axes of low-mass protostars can be identified with some precision. Often, observations indicate the presence of a disk around the protostar, presumably an accretion disk in which gas and dust spiral inwards and onto the rotational equator of the protostar. And in many cases matter is being blown out in a supersonic jet along the two poles of the rotation axis, boring a hole through the surrounding interstellar medium, and pushing ahead great blobs of molecular gas. The resulting system of shock waves produces chains of Herbig–Haro objects sometimes terminating in a clearly defined bow shock (see also the recent News and Views article by Hartmann in *Nature* **340**, 432; 1989).

Modelling of protostellar accretion disks is still elementary, but typically they have radii of about 5×10^{14} m and masses of 0.1–1.0 solar masses. Most of the uncertainty in the mass derives from our poor knowledge of the far-infrared emissivity of protostellar dust (J. Emerson, Queen Mary College, London).

* Low-Mass Star Formation and Pre-Main Sequence Objects, ESO Workshop, 11–13 July 1989, Munich.

Ultimately, some (and maybe all) protostellar disks will presumably develop into planetary systems. From general considerations it can be shown (G. Morfill, Max-Planck-Institut, Munich) that the variations in radial temperature and surface density that were required to produce the different masses, elemental compositions and formation timescales of the planets in the Solar System arise naturally in protostellar disks.

But fundamental problems remain. We are still very far from understanding why most stars are in binary and multiple

systems. There are compelling theoretical reasons to believe that binary formation is a highly non-quasistatic process (J. Pringle, Cambridge University). If this reasoning is correct, then it is very difficult to see how the quiescent, centrally condensed protostellar cores modelled by Lizano can become binaries and we are forced to look to some other site for binary formation. □

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GENE REGULATION

Action of leucine zippers

Ted Abel and Tom Maniatis

A FUNDAMENTAL question in gene regulation is how a finite number of regulatory factors and DNA-binding sites can generate the enormously complex pattern of tissue-specific gene expression required for development. Steve McKnight and colleagues provided a new way of thinking about this problem last year when they discovered the 'leucine zipper' — a stretch of 35 amino acids containing 4–5 leucine residues separated from each other by 6 amino acids — in various DNA-binding proteins. They proposed that the leucines are arranged on one side of an amphipathic α -helix and that the leucines on two such helices would interdigitate leading to dimerization. Noting that the zipper was immediately preceded by a region rich in positively charged amino acids, they further suggested that dimerization would arrange the basic regions of the two sub-units in a configuration that allows specific interactions with a DNA-recognition sequence (see figure), a model that was soon confirmed in many laboratories. The 'domain swap' experiments reported recently by Kouzarides and Ziff¹ and by Sellers and Struhl on page 74 of this issue² have particularly interesting implications for combinatorial models of gene regulation.

The leucine zipper was originally identified in a site-specific DNA-binding protein known as C/EBP. A computer search³ revealed a similar repeat in the amino-acid sequences of Fos, Jun and Myc, nuclear proteins encoded by proto-oncogenes, and in the yeast transcription factor GCN4. Meanwhile, evidence was accumulating for a role of the *fos*-gene product in the control of gene expression during adipocyte differentiation, and a Fos-related protein was identified as the product of the *c-jun* oncogene (reviewed in refs 4,5). Also, the c-Jun protein was shown to be a member of the AP-1 family of transcription factors. A series of experiments subsequently demonstrated that the Fos and Jun proteins form a hetero-

dimer that binds to the AP-1 or TRE (TPA-responsive element) regulatory sequence in certain genes and activates transcription. The Fos-Jun heterodimer binds the TRE with high affinity, and is a strong transcriptional activator; the Jun homodimer binds more weakly and is less potent. The Fos protein alone does not dimerize or bind DNA. GCN4 binds to a yeast regulatory sequence that is identical to TRE, but it does so as a homodimer.

As suggested by the leucine-zipper model, both C/EBP (ref. 6) and GCN4 (ref. 7) dimerize and recognize palindromic sequences. Direct evidence that the zipper creates a dimerization interface between protein subunits was provided by structural studies of a synthetic peptide corresponding to the leucine-zipper region of GCN4 (ref. 8). Circular dichroism and two-dimensional NMR studies revealed that these peptides associate to form very stable dimers of α -helices. McKnight and colleagues originally proposed that these helices would associate in an antiparallel fashion, which would allow the long leucine sidechains to interdigitate

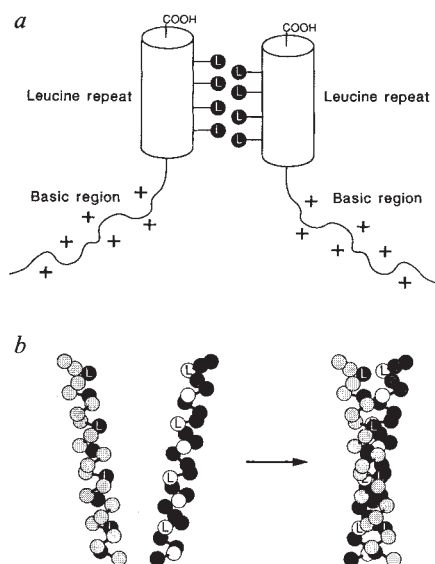
Diagrams showing structural features of the leucine-zipper motif present in several DNA-binding proteins. *a*, The bipartite DNA-binding domain of C/EBP (ref. 1). The leucine repeat region, which could form an eight-turn α -helix (drawn as a cylinder), serves as a dimerization interface. Dimerization is necessary for specific interactions between the adjacent basic regions and the DNA-recognition sequence. *b*, The coiled-coil model for the structure of leucine zipper⁸ (modified from ref. 24). The α -helical regions of two monomers are illustrated on the left; each residue is represented by a sphere centred on the C α atom. In the dimer (right), these helices form a coiled coil, a structure in which two parallel helices are intertwined. The leucine residues are indicated by black or white balls marked with an L, and the alternating hydrophobic repeat (present in many, but not all, leucine zippers) by plain black or white balls. These two interspersed heptad repeats, known as the 4–3 repeat, form the interaction surface between the two helices.

"like the teeth of a zipper". Cross-linking studies⁸, however, demonstrated that the helices of the leucine zipper region of GCN4 are parallel in the dimer, a conclusion supported by analyses of the effects of specific leucine substitutions on dimerization of C/EBP (ref. 6), Fos and Jun⁹.

The leucine zipper of GCN4, therefore, seems to have the characteristics of a coiled coil, found in many fibrous proteins such as keratins, lamins and myosin¹⁰. The structure of such coils, originally proposed by Francis Crick¹¹, is a left-handed twisting of two α -helices about each other (*b* in the figure). Classically, the physical basis of this quaternary structure is provided by a 4–3 repeat of hydrophobic amino acids that lie along the face of the α -helix. Efficient 'knobs-into-holes' packing of these apolar side chains occurs at the interface between the two helices. The heptad repeat of hydrophobic amino acids interspersed within the leucine repeats of GCN4, Jun and C/EBP could give the same coiling. It appears, therefore, that the leucine sidechains may not interdigitate, but rather they interact with the alternate, non-leucine, hydrophobic residues in the opposing helix⁸.

The leucine zipper regions of Myc and Fos, however, do not contain hydrophobic residues at most of the alternate positions. The ability of Fos to dimerize with Jun could, therefore, be a consequence of different types of interactions at the helix interface. For example, the methylene groups of a lysine side chain at one of these positions provide an alternative hydrophobic contact. Electrostatic effects could also act to stabilize heterodimers of Fos and Jun¹². The role of these novel interactions in determining the specificity of cross-dimerization remains to be established.

Other transcriptional regulatory proteins, such as the *Drosophila* zeste protein and the lymphoid cell-specific octamer-binding protein (OTF-2), have potential



leucine-zipper domains, but they are not required for binding to known DNA-recognition sequences¹³. These domains could be required for interactions with other proteins or with DNA-recognition sequences not yet identified. The leucine zipper has also been identified in certain types of membrane proteins, including a family of voltage-gated potassium channels¹⁴, glucose-transporter proteins¹⁵ and the F glycoproteins of paramyxoviruses¹⁶. In each case, however, the zipper is not adjacent to a basic region, so that the leucine repeat may be an independent structural domain used by diverse families of proteins for specific protein-protein interactions.

The combination of a basic domain and a leucine zipper in Fos, Jun, GCN4 and C/EBP indicates that they represent a novel class of DNA-binding proteins. Recent mutagenesis experiments have precisely defined the dimerization and DNA-binding motifs of proteins in this family. When leucine residues in the zipper region are altered, even to other hydrophobic amino acids, the proteins neither dimerize nor bind DNA^{5,6,9,17,18}. By contrast, mutations in the basic region prevent DNA binding, but do not interfere with dimerization^{5,6,9,17}. These observations strongly support the notion of separate dimerization and DNA-binding domains originally proposed by McKnight and colleagues¹.

The new domain-swap experiments of Kouzarides and Ziff² and Sellers and Struhl³ further explain the independent functions of these domains. By placing the Fos basic region next to the zipper of GCN4, both groups show that the basic region of Fos can specifically bind to the TRE if present as a dimer. Although the native Fos protein cannot form a homodimer and binds DNA only by forming a Fos-Jun heterodimer, the Fos/GCN4 chimaeric protein forms a homodimer that interacts specifically with the TRE. The basic regions of Fos, Jun and GCN4, therefore, are equivalent in their DNA-binding abilities, indicating that their differential affinity for the TRE results from the differing ability of their leucine zippers to form dimers.

Leucine-zipper proteins do not dimerize randomly: GCN4 and C/EBP form homodimers, whereas Fos and Jun participate in heterodimeric complexes. Kim and co-workers have recently shown¹² that short peptides containing the leucine-zipper regions of Fos and Jun preferentially form heterodimers of parallel α -helices. Kouzarides and Ziff investigated² the basis for selective dimer formation by fusing the Fos zipper to the GCN4 basic region. Unlike GCN4 itself, this chimaeric peptide forms a complex with Jun, and the heterodimer binds specifically to the TRE, indicating that the leucine-zipper regions of Fos, Jun and GCN4 are both

necessary and sufficient to determine the selectivity of dimer formation. The structural basis for this selectivity is not yet understood. But because all zipper proteins contain the leucine repeat, other amino acids in this region must play an essential role. Substitution of single non-leucine residues has no effect on dimerization, but this may indicate that multiple interactions along the faces of the helices contribute to the stability of the dimer^{17,18}. A systematic series of regional swaps or multiple amino-acid substitutions will be required to identify the molecular basis of the specificity.

All heterodimers formed between the leucine-zipper proteins studied so far, bind to the same DNA sequence. But heterodimer formation could result in novel DNA-recognition properties, or in different regulatory activities, thus expanding the repertoire of a finite number of transcriptional activator proteins. The demonstration that a heterodimeric repressor protein can bind to a hybrid operator sequence¹⁹ is a precedent for the notion that a heterodimer can bind to a sequence not recognized by either homodimer. The basic regions of Fos, Jun and GCN4 recognize the same DNA sequence, but C/EBP recognizes an entirely different sequence. It would be interesting to determine whether the juxtaposition of two different basic regions in a heterodimer of C/EBP and Fos could generate a novel DNA-binding specificity. The possibility that different protein complexes display distinct transcriptional regulatory activities is indicated by the observation that Fos can act as a positive or negative regulatory protein in different DNA-sequence contexts. Fos, for example, seems to stimulate transcription when bound to the TRE, but acts as a negative factor when bound to an adipocyte-specific regulatory element^{4,5}. Fos also negatively regulates its own expression^{4,5}.

The formation of heterodimers may also be important in the function of an entirely different set of transcriptional activator proteins, the Myc/MyoD or helix-loop-helix family²⁰. These proteins interact to generate complexes that bind a specific DNA sequence with a higher affinity than the individual protein subunits²¹. E47 and E12, for example, are proteins that bind to the immunoglobulin- κ -chain enhancer as dimers; MyoD is a transcriptional activator that converts fibroblasts to myoblasts, and which seems to act by turning on muscle-specific gene expression. These proteins form heterodimers that bind to nearly identical sequences present in both the immunoglobulin and muscle-creatine-kinase enhancers²¹. The formation of heterodimers in this case requires a novel dimerization motif within the helix-loop-helix motif²¹. Interestingly, at least two members of the Myc/MyoD family, E12 (ref.

20) and myogenin²², also contain a leucine repeat. Thus, interfamily dimerization may be possible.

Pairwise association of proteins through two distinct sets of dimerization domains could also lead to the formation of a virtually unlimited variety of multicomponent complexes. Members of the helix-loop-helix family of proteins include tissue-specific factors such as MyoD and two other myogenic regulatory proteins, as well as *Drosophila* genes that are involved in neurogenesis, such as *achaete-scute* and *daughterless*²⁰. Other members of this family, such as E47, however, are expressed in all cell types, indicating that tissue-specific gene expression could be the result of novel combinations of heterodimers.

The finding that leucine zippers are responsible for specific heterodimer formation provides a conceptual framework for understanding combinatorial models of gene regulation. Transcriptional regulatory proteins consist of DNA-binding and transcriptional activation domains which are interchangeable between individual proteins as well as among widely divergent organisms²³. However, the complexity involved in achieving the highly specific pattern of gene activation required for the growth and development of eukaryotic organisms is staggering. This specificity may be achieved, at least in part, by novel protein-protein interactions made possible by the dimerization of regulatory proteins. □

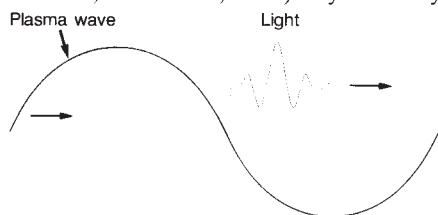
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Surfing for light waves

A. R. Bell

THE diversity of nonlinear interactions involving waves in plasmas is both a fascination and a frustration to plasma physicists. Often the interactions arise unexpectedly to frustrate attempts to design useful plasma devices, but the very wealth of interactions gives great scope for inventive ideas. The latest ingenious idea springs from the University of California at Los Angeles where S. C. Wilks, J. M. Dawson and co-workers have recently suggested that it may be possible to 'accelerate' photons in a plasma (*Phys. Rev. Lett.* **62**, 2600–2603; 1989). By this they



Schematic of the photon accelerator, showing the electron plasma wave, with the wavefront moving close to the speed of light and boosting the frequency of the light pulse.

mean that photons initially with low energy can be changed into high-energy photons as they pass through a suitably excited plasma.

Behind the concept is the hope of producing a high-frequency, coherent electromagnetic wave by upshifting a low-frequency coherent wave — an alternative to direct laser action at the higher frequency. The idea presents an enticing prospect. Coherent high-energy radiation is not easily produced, especially when X-ray energies are approached. The proposed scheme is similar to those currently being investigated for accelerating charged particles — for example the plasma beat-wave accelerator (PBWA). Indeed, Dawson was, with T. Tajima, one of the originators of the beat-wave and related ideas (*Phys. Rev. Lett.* **43**, 267–270; 1979).

In either scheme, a large-amplitude wave is generated in an ionized plasma by intense laser beams or by an energetic particle beam. The wave consists of a sinusoidal variation in electron density which moves with a phase velocity slightly less than the speed of light.

For simplicity, view the process in the reference frame moving with the plasma wave. In this frame the sinusoidal density variation is stationary. In the photon accelerator, a short pulse of electromagnetic radiation (short compared with the wavelength of the plasma wave) can be trapped in one of the low-density troughs of the wave, because electromagnetic radiation of a given frequency cannot propagate above a certain density (the critical density). The density trough can

be seen as behaving like a potential well for the photons comprising the electromagnetic pulse. The group velocity of an electromagnetic wave propagating up a density ramp reduces as the density increases, until it becomes zero at the critical density where it is reflected down the density gradient. A pulse initially placed at the bottom of the trough and travelling backwards with respect to the wave will climb the wall of the trough, reverse its direction when it reaches the critical density and returns to its original position with its original frequency and energy. Its group velocity is unchanged in magnitude but reversed in direction.

Transforming back into the laboratory frame, we see that because of the change of direction, the magnitude of the laboratory group velocity has in fact increased: the pulse starts at the bottom of the trough, moving in the same direction as the plasma wave but at a slightly lower velocity (a suitable choice of light frequency gives the required group velocity). The pulse initially drops back relative to the wave, but then accelerates to a velocity higher than that of the plasma wave, eventually returning to the bottom of the trough with an increased group velocity. Because the group velocity has increased, so have its frequency and photon energy — the photons have been accelerated.

The analysis by Wilks *et al.* shows that the basic scheme can increase the electromagnetic frequency by a factor of around 5, although this requires a plasma wave in which the excursion of the electron density from its mean is half the mean density. This amplitude is much larger than that of any coherent plasma wave created so far by proponents of the PBWA, but it is not impossible given the rapid developments in this field. To obtain a larger frequency upshift or to manage with less extreme plasma waves, Wilks *et al.* suggest steadily raising the plasma density along the length of the accelerator.

Many problems still need to be addressed. For example, a suitable means of driving a plasma wave with the required amplitude, coherence and phase velocity needs further investigation. The ultimate efficiency of the system is uncertain. Undesirable wave-wave interactions could compete with those desired for photon acceleration. And problems could arise when the present one-dimensional analysis is replaced by a full two- or three-dimensional one. The proof of the theoretical pudding will be in the experimental realization. □

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Bubbling upwards

LAST week Daedalus invented a solution for blowing really robust soap bubbles. It contains big closed-loop polymer molecules: any small puncture is certain to be surrounded by at least one loop molecule. So it cannot spread and is soon healed by brownian motion. Invulnerable to the usual mode of failure, a 'Hyperbubble' is a wonderfully light and robust structure, rich with technological promise.

Daedalus is directing this promise firmly upwards. He points out that a traditional balloon is only partly filled with gas before its release. As long as its envelope is slack, the pressure inside and outside are equal and the lift of the balloon remains constant. But as it rises through the thinning air, it expands steadily and ultimately fills taut. The balloon has reached its ceiling: any further ascent reduces its lift.

But a Hyperbubble would behave quite differently. Such a stable soap bubble would simply expand indefinitely as it rose and would pull taut only when it had thinned to a perfect monolayer. Daedalus calculates that a hydrogen-filled Hyperbubble with an initial radius of a mere metre or so, and an initial film thickness of 0.1 mm, could lift itself from sea level and reach about 160 km altitude. In practice, of course, the bubble would have the usual 0.001 mm soap-film thickness even at ground level, but could carry the extra liquid in a reservoir to top-up the thinning film as it expanded at higher altitudes.

Attaching a payload to a big liquid-skinned balloon poses intriguing problems. Daedalus hopes that a complex corded harness, light and fairly flexible, can be given such an extended line of wetted contact with the bubble that the total surface tension along the line will support a few kilograms of instruments. A space-going Hyperbubble cannot contain water, which would evaporate rapidly in the low pressure. Liquid polymers, high-molecular-weight detergents and other novel soap-film components are being investigated. But ultimately Hyperbubbles should lift research equipment up to 100–200 km amazingly simple, cheaply and reliably.

At operating altitude a Hyperbubble will be a vast attenuated object drifting before whatever vacuous molecular wind there is up there. Though immune to punctures from micrometeorites, it will still slowly lose gas by diffusion, and gradually descend. In the process it will slowly shrink and thicken, at each stage forming an effective parachute for the local atmospheric density. Cosmic-ray film packs, survey cameras, magnetometers, all will be lifted gently and cheaply into near space and as gently returned. The robustness and reliability of a Hyperbubble would only really be overwhelmed by collision with an orbiting satellite.

David Jones

Vent fauna on whale remains

SIR—Concentrations of animals supported at least partially by chemoautotrophic production are known from a variety of geologically produced reducing environments at the deep-sea floor^{1,6}. During DSRV *Alvin* dive number 1,949 (10 November 1987) in the 1,240-m deep Santa Catalina Basin (33°14'N, 118°30'W) we discovered a chemosynthetic community fuelled by a novel source of reduced compounds — the intact skeleton of a 20-m long whale. Data collected during subsequent dives (numbers 2,132–2,138, 5–11 November 1988) indicate that the partially buried remains of this blue or fin whale have produced a microhabitat distinct from the surrounding, well studied^{7–9} basin floor.

Many of the larger whale bones and nearby patches of sediment were covered with white microbial mats reminiscent of those at some hydrothermal vents^{10,11} and petroleum seeps². The mats were composed of a diverse assemblage of colourless sulphur bacteria, including large filaments (100 µm wide × 2–3 cm long) most closely related to *Beggiatoa gigantea*¹². Recovered whale bones were laden with oil and smelled strongly of sulphide, and the pore water of underlying surface sediments had sulphide concentrations of up to about 20 µM (measured by B. Hales with sulphide electrodes on recovered cores), indicating that the chemical reducing power supporting these bacterial mats is derived from bone-oil seepage. In dozens of previous submersible dives and camera surveys at the Santa Catalina Basin floor^{7–9}, we had never before observed *Beggiatoa* mats.

The whale skeleton also harboured six metazoan species not encountered before in the Santa Catalina Basin (see table). Among these were two species of large vesicomyid clams, *Vesicomya gigas* and *Calyptogena cf. pacifica*, which nestled in bone crevices and dotted sediments near the whale. We observed more than 50 living individuals and hundreds of shells, ranging from 2 to 10 cm in length. Scoop net samples suggested that most of these clams live hidden in the sediment near the skeleton.

Other atypical basin species included the mussel *Idasola washingtonia*, an

undescribed coccinid limpet and the snail *Mitrella permodesta*; all three were abundant on exposed bone surfaces with densities of >100 m⁻². We also collected the bivalve *Lucinoma annulata* from small cavities in the skeleton, and from adjacent sediments.

Several of these species are characteristic of reducing habitats in the deep north-east Pacific (see table). *Vesicomya gigas*, (misidentified in refs 13 and 14 as *Calyptogena pacifica*; R. D. Turner, personal communication) occurs in abundance at Guaymas Basin and Juan de Fuca vent fields (ref. 13 and R. D. Turner, personal

communication), possibly at Juan de Fuca vents (V. Tunnicliffe, personal communication), and widely on sunken wood¹⁹. Transmission electron micrographs and enzyme assays indicate that *I. washingtonia*, but not *M. permodesta*, hosts chemoautotrophic bacteria (J.W.D. *et al.*, manuscript in preparation).

The occurrence of a chemosynthetic community on the whale bones appears not to be an isolated incident; endosymbiont-containing *I. washingtonia*, as well as *M. permodesta* and pyropeltid limpets (a family known previously only from vents²⁰), have recently been found on whale bones trawled from three other slope sites off California (W. Wakefield,

TABLE 1 Distribution of animal species associated with the whale skeleton not normally found in the Santa Catalina Basin

Species	Vent/seep collection sites	Other collection sites	
		Locality	Habitat type
<i>Vesicomya gigas</i> *	Guaymas Basin, Juan de Fuca Ridge	Gulf of California	vents?
<i>Calyptogena pacifica</i> *	San Diego Trough fault, Guaymas Basin? Northern California, Juan de Fuca Ridge?	Alaska slope	?
<i>Lucinoma annulata</i> *	—	California Basins	anoxic sediment
<i>Idasola washingtonia</i> *	Guaymas Basin, Juan de Fuca Ridge?	Washington slope	?
		Southern Japan slope	sunken wood
		New Zealand slope	sunken wood
		Central California slope	whale bones
<i>Mitrella permodesta</i>	—	California Basins	anoxic sediment
Coccinid limpet	—	—	—

* Known to contain chemoautotrophic endosymbiotic bacteria.

communication), and hosts large populations of endosymbiotic chemoautotrophic sulphur bacteria (based on transmission electron microscopy and enzyme assays; J.W.D. *et al.*, manuscript in preparation). *Calyptogena pacifica* contains similar endosymbionts and occurs at many sites, including vents and a cold seep^{15–17}. *Lucinoma annulata* also obtains nutrition from chemoautotrophic endosymbionts¹⁵, and occurs in abundance near sewage outfalls off California¹⁸. Members of this genus are also found in petroleum-seep communities of the deep Gulf of Mexico¹⁴. Finally, *M. permodesta* abounds in anoxic sediments in some Californian basins (J. McLean, personal communication), and *I. washingtonia* has been collected at

personal communication).

In retrospect, it is not surprising that a whale carcass on the energy-poor deep-sea floor should produce a community with ecological and taxonomic similarities to other deep-sea reducing habitats. Microbial degradation of organic-rich whale remains appears certain to yield sulphide from sulphate reduction²¹ and putrefaction²². The consequent occurrence of sulphides with a nearby source of oxygen (in bottom water) should provide a habitat analogous to seeps and vents in its suitability for sulphide-oxidizing bacteria and their metazoan hosts^{13,15}.

These findings suggest that whale skeletons might serve as important 'stepping stones' for the dispersal of deep-sea

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animals that depend on chemosynthesis. For that to be the case, they must at least provide reductants for long enough to allow chemosynthesis-dependent species to reproduce — more than five years for many vent species¹³ — and be common in space relative to other reducing habitats.

If the large (up to 10 cm) vesicomyids on and around the whale bones grow at the high rates measured for hydrothermal-vent vesicomyids¹³, the reducing habitat must have been present for at least 3–17 years. Assuming, conservatively, that whale skeletons support chemosynthesis for five years in the deep sea, and that only half of the approximately 1,000 adult grey whales (*Eschrichtius robustus*) that die each year in an 8×10^5 -km² area of the north Pacific²³ sink to, and remain on, the sea floor (also a conservative estimate; D. W. Rice and D. Withrow, personal communication), there would be approximately one reductant-rich skeleton per 300 km². If these carcasses were randomly distributed over the grey whale range, the average distance between nearest neighbours would be about 9 km (ref. 24). In fact, deaths and sinking carcasses seem highest along migration routes between Alaskan waters and the Gulf of California^{24,25}. These rough but probably conservative calculations suggest that whale skeletons may indeed provide persistent and abundant stepping stones for dispersal of deep-sea chemosynthetic communities over large areas of the north-east Pacific.

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Greenhouse dust

SIR—Recent concern expressed by the scientific community and by ecologists over anthropogenic 'greenhouse gases', notably CO₂, has been attended by a neglect of other factors that contribute to a fluctuating background level of atmospheric (surface) temperature¹. It is well known that the mean surface temperature can fall by amounts ranging from a few tenths of a degree to a degree or more in extreme cases immediately following a major volcanic eruption, and that the effect can persist for a year or two^{2,3}. For example, the Tambora eruption of 1815 led to the 'year without summer' and smaller effects are clearly observed in more recent volcanic events, including the Mount St Helens eruptions of 1980.

Whether the surface temperature falls or rises following an injection of volcanic particles, and by how much, is a complex issue depending on, among other factors, the particle size distribution and bulk optical properties⁴.

Another important background effect could result from the accumulation at the Earth of extraterrestrial dust particles — interplanetary dust including Brownlee particles (a subset of interplanetary dust particles sampled in the Earth's upper atmosphere) and particles arising from direct encounters with small icy comets. These particle inputs are all highly variable as well as uncertain. The calculations that follow are therefore illustrative, but not definitive. The Brownlee-particle influx, estimated from direct collections of ~ 10 - μ m-sized particle clusters, may be $\sim 10^{10}$ g yr⁻¹ (using data in ref. 5), but a submicron component, which is expected to be present, could result in a higher total flux $\sim 10^{11}$ g yr⁻¹. With an estimated stratospheric residence time of ~ 10 yr for the smallest grains we could reasonably expect an average load of $\sim 10^{12}$ g of extraterrestrial particulate material, giving a surface density of $\sim 2 \times 10^{-7}$ g cm⁻² over the entire Earth.

For the highly porous silicate or organic particles of radii $\sim 10^{-5}$ cm expected from comets and micrometeorites, a typical mass extinction coefficient as a result of scattering at visible wavelengths is of the general order of 10^5 cm² g⁻¹ (ref. 6). This is about 10–100 times the value of the mass absorption coefficient for silicates or organic solids at their infrared absorption peak near the wavelength of ~ 10 μ m. For a surface density $\sim 2 \times 10^{-7}$ g cm⁻² of such particles, which are effectively 50 per cent backscatters, an effective optical depth for attenuation of visible sunlight available for heating the surface is $\sim 0.5 \times 10^5 \times 2 \times 10^{-7} \approx 0.01$, whereas their contribution to heating through infrared absorption will be negligible.

In view of the highly variable nature of the extraterrestrial particle load a typical fluctuation of this effective optical depth will also be of the same order, $\Delta\tau \approx 0.01$. The solar input for heating could thus fluctuate by $\Delta F/F \approx 1 - e^{-\Delta\tau} \approx 0.01$, which corresponds to a temperature fluctuation $\Delta T/T \approx \Delta F/F \approx 0.25$ per cent. Because temperature fluctuations of about this magnitude are being discussed in connection with the greenhouse problem,

we suggest that a proper assessment of the matter must include a consideration of processes of the type discussed above. Small decreases in the background load of extraterrestrial dust would give rise to apparent increases in global temperature. Large increases of this load, which would occur on rare encounters with large comets, could lead to ecodisasters^{7,8}.

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Cold fusion and brown dwarfs

SIR—Jones *et al.*¹ claim to have observed neutrons resulting from cold nuclear fusion of deuterons within the palladium metal lattice. They state that observation of naturally occurring ³He in the Earth (again attributed to cold p–d fusion occurring in geophysical environment at a rate of $\sim 10^{-24}$ fusions per d atom per second) and the excess luminosity radiated by Jupiter suggested to them new directions for laboratory studies of cold nuclear fusion.

For instance, the 10^{18} W radiated by Jupiter could be produced by p–d fusions at a rate $\lambda_f \approx 10^{-19}$ fusions d⁻¹s⁻¹. Recently, objects intermediate in mass between Jupiter and a typical star, brown dwarfs, have also been attracting attention; some may have been detected². An object about 20 times the mass of Jupiter ($20M_J$) would have a central density ρ_c (which for such objects, with near degeneracy at their cores, scales as M^2) about 400 times larger. An object of $30M_J$ would have $\rho_c \sim 900$ times larger, and so on. Their central pressure scales as $M^{10/3}$ and would then be much larger than that of Jupiter. So, for a similar p–d composition (which one can reasonably assume for unevolved stellar objects), such objects would have a cold fusion rate ρ_c^2 —times larger (the product of number densities of p and d) than Jupiter. A $20M_J$ brown dwarf undergoing p–d cold fusion would radiate $\sim 1.6 \times 10^{22}$ W or nearly 10^{-4} of the solar luminosity. But from their extreme faintness such objects are deduced to emit only 10^{-7} solar luminosity or less (even for an estimated $50M_J$ object which should be much brighter, with 6×10^{-13} fusions d⁻¹s⁻¹). One can reasonably conclude that Jupiter is not getting its luminosity from such reactions which should have made brown dwarfs appear several magnitudes brighter.

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A fitting endeavour

Chris Brand

Genes, Culture and Personality: An Empirical Approach. By L. J. Eaves, H. J. Eysenck and N. G. Martin with contributions from R. Jardine, A. C. Heath, L. Feingold, P. A. Young and K. S. Kendler. *Academic*: 1989. Pp.465. £32.50, \$65.

"THE post-1968 New Left in Britain and the United States has shown a tendency to see human nature as almost infinitely plastic, to deny biology and acknowledge only social construction." So much was admitted by Rose, Kamin and Lewontin, the left-wing co-authors of *Not in Our Genes* (Pantheon, New York/Penguin, London; 1984), as they nevertheless told psychologists to acknowledge complex biological influences on development lest the vacuum of New Left environmentalism should encourage old-fashioned biological determinism and simplistic hereditarianism. The present co-authored volume, in preparation over the past 15 years, is a much needed attempt to deliver the required psychogenetic sophistication in accounting for human personality differences.

Essentially, Eaves *et al.* indicate the possibilities of tracing questionnaire scores to at least some of the sources of variance envisaged by the population geneticist: to additive genetic factors that make closer relatives more similar, to 'between-family' environmental differences such as social class, to 'within-family' origins of differences between siblings, to assortative mating by parents that maintains extreme values of traits in the population, and the rest. To realize such possibilities, the authors repeatedly fit various models, involving varying hypothetical values for such parameters, to data from large twin and adoption studies conducted in London, Sweden and Australia.

Necessarily, the book is replete with tables and formulae; but, as champions of the non-simplistic might expect, really convincing conclusions are hard to find. The authors' main conclusion, however, which has become conventional wisdom amongst psychogeneticists in the 1980s, is clear. It is that families have few general environmental ('between-family') effects on the personalities of children reared in them — quite contrary to popular, and probably to 'New Left' belief. At least, most families' children vary widely in 'neuroticism', 'extraversion' and 'psychoticism'; the admittedly greater similarities between siblings in social attitudes may be attributable to assortative mating; and the well-known (though slight) effects of family environment on IQ do not concern the present authors.

Most readers, however, will find it hard to be persuaded of even that main

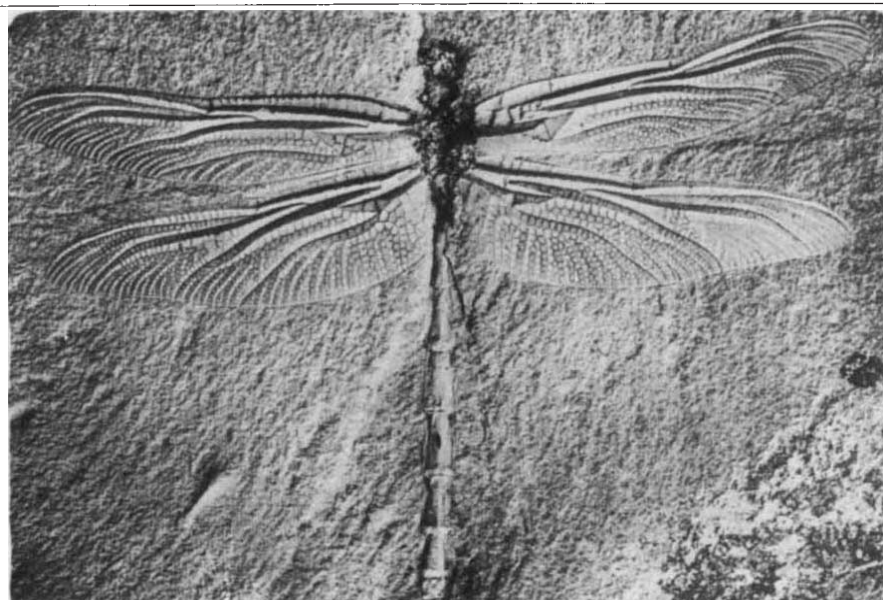
conclusion of the book, until they reach page 127. Here, a revealing table sets out the astonishingly low correlations that the authors find not only between foster siblings, between nieces and aunts, and between fathers and sons, but even between fraternal twins who grew up together. For example, male fraternal twins in these London data correlate on average at only +0.07 across four different personality measures. Here, at last, there is little need for formulae and models to explain what is happening. Yet the authors prefer to press ahead with their modelling exercises rather than discuss the immediate implications of their remarkable raw data. Equally strangely, the table does not provoke the authors to consider that their only strong correlations (of around +0.53, between male identical twins) cry out for explanation in terms of epistasis (gene-gene interaction) which predicts substantial similarities between relatives only if a large percentage of relevant genes is held in common.

Why do Eaves and his colleagues tend to hide behind their formulae and models, and decline to provide compelling discussions of data even when opportunity

knocks? One problem, admitted on the penultimate page of the book, is that their methods and data simply do not allow the handling of putative genetic-environmental interaction effects. Nor can readers be given much indication as to whether genetic-environmental covariation is at work. So the authors may have deemed it better to press on with fitting such models as they could. Another problem is the unrepresentativeness of their subjects — many of the London twins were recruited via a particular David Frost TV programme, for example.

Yet even with the help of volunteer samples of twins — which are bound to over-represent phenotypically similar identical twins who will understandably have more interest in twin research — the authors are only claiming a modest acquaintance with the causation of variables selected over some 40 years (in Hans Eysenck's programme of work) as seeming capable of yielding to scientific endeavour. What has happened here is that proponents of *nomothetic*, scientific approaches to human psychological differences are losing, at half time, by their own scrupulosity, to their classic opponents, the champions of *idiographic*, individuality-acknowledging approaches to personality. For the present, only IQ is left as a variable that behaves like a paradigmatic nomothetic personality trait — yielding greater phenotypic similarities in closer relatives. In contrast, much of the rest of adult personality now appears to emerge by magic.

These authors' evasiveness and compulsive model-fitting constitute one



On the wing — *Protolindenia wittei*, a fossilized true dragonfly with a wingspan of more than 10 cm. (Jurassic, Solenhofen, Germany.) Insect wings are thought to have developed from flaps on the first three thoracic segments; insects fully equipped for flight have been found as early as the Lower Carboniferous. Some species of ancestral dragonflies had wingspans of 70–76 cm. Like true dragonflies, they were swift flying predators with spiny legs used in capturing prey. The picture is taken from *The Fossil Book: A Record of Prehistoric Life* by Carol Lane Fenton and Mildred Adams Fenton, published by Doubleday.

legitimate response to the undermining of simple determinism about personality; but there is another. Conceivably, Eaves *et al.* might dispute the arguably modish terminology of 'transaction', 'emergence' and 'chronogenesis' which some writers in the 1980s have more daringly used to begin to describe how genes, in combination, might do their 'magic' (partly environment-shaping) work during psychological development. As it stands, however, the book contains little discussion of the work and theorizing of Sandra Scarr, R. J. Rose, Robert Plomin, David Lykken and Tom Bouchard. This unwillingness to join the 1980s hunt for an explanation of within-family variance is a disappointment — reflecting partly, of course, the book's long gestation period and the degree of international co-operation that was involved.

Nevertheless, the authors have produced a scholarly fulfilment of the best hopes of *Not in Our Genes* and can reasonably hope to provide more engaging theoretical analyses in future with the help of their substantial databases. Simple hereditarianism has at least proved easily superior to simple environmentalism; and more complex genetic effects are set fair to pick off the rest of personality variance. □

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Genetics of Neuropsychiatric Diseases

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In the search for genetic links to schizophrenia and other etiologically unclear neuropsychiatric diseases, it is useful to learn how genes have already been mapped and genetic defects discovered in some related diseases. *Genetics of Neuropsychiatric Diseases* includes examples of both.

Also included are a gene map of neuropsychiatric disorders and diseases, enzymes and proteins of neuropsychiatric interest, as well as reports on DNA techniques and DNA linkage, and chromosomal aberrations as tools for gene mapping.

The book also discusses the research findings and clinical practice of several diseases. New techniques and the recent finding of a gene segment linked to schizophrenia are reviewed.

April 1989 550.00 378pp 234x156mm
0-333-48891-1

This book is available from your usual bookseller or from: Dionne Stocking, Macmillan Press Ltd, Houndmills, Basingstoke

On the darker side

John Dunster

Multiple Exposures: Chronicles of the Radiation Age. By Catherine Caufield. Secker & Warburg, London/Harper & Row, New York: 1989. Pp. 304. £12.95, \$19.95.

THE first thing to say about *Multiple Exposures* is that it grips. I found myself over half-way through at my first sitting with no more than trivia on my note-pad — the initial account of the discovery and early applications of X-rays and radioactivity combines detail and interest in a masterly way.

Most of the rest of the book is based on American experience, with occasional forays into the international scene. The emphasis is on the building and testing of nuclear weapons and the developing use of radiation in medicine, the author stepping back at intervals to look at the formulation of radiation-protection principles and policy. The selection is a little capricious. Nuclear electricity and nuclear propulsion of warships are barely mentioned, and then only in relation to accidents, and the use of radiation in industry and research is ignored. Nevertheless, the story is told well and accurately; although there are errors, most of them are harmless and do not detract from the main theme.

Perhaps the main weakness of this book is its principal title. Not until Chapter 20 does the idea of multiple exposures appear, and it never has more than a minor part. In fact, the system of radiological protection is specifically aimed at taking account both of single sources and multiple exposures (would that the control of toxic chemicals could be as effective!). All of the examples of failure in the book result from inadequate protection for single sources.

One of the book's important contributions is brought out first in the preface, in which Caufield clearly presents the perennial problem of conveying precise scientific information to the public without sounding either shifty or overconfident. She understands well the problems of setting standards for hazardous agents that may have no level of exposure that is totally risk-free, and also demonstrates the human capacity for letting blind belief and self-interest triumph over observation. Her quotations from the early enthusiasts for the benefits of X-rays and radium are chilling. But any sense of modern superiority can be dispelled by a visit to the local chemist's shop with a call at a health-food store on the way. Blind faith is still with us, though the dangers

seem to be more limited.

In the third and fourth parts of the book, my initial enthusiasm became tempered by two concerns. The first was the selective nature of the "Chronicles". History is always selective, and authors of good historical books declare the basis of their selection. Caufield doesn't. Her choice of examples seems to be aimed at identifying only the harm done by radiation and by the practices that use or produce it. There is nothing wrong in that, but it would have been better to acknowledge it.

My second concern is more serious. Whenever a quotation emphasizes the dangers of radiation, it is subtly enhanced. Comments that might reassure are made to sound biased. In its worst form, this tendency takes the form of hinting that anyone who makes a statement about radiation risks or standards that is not hostile to governments or industries is deliberately supporting those governments or industries. Despite her understanding of the need to balance the risks to those exposed against the benefits to society, the author is unwilling to recognize that some people might oppose tighter standards because they believe that further restrictions would genuinely do more harm than good. One of the hard facts of life in work on safety and health protection is that all protective measures do some harm as well as some good. Increasing the good, considered in isolation, is easy if money is no problem, but getting the right balance between good and harm is very difficult indeed. Where limits are concerned, lower is not always better, and virtue is not confined to those who oppose the established views.

A book review is no place for me to defend either the International Commission on Radiological Protection (ICRP), of which I am a member, or the National Radiological Protection Board (NRPB), of which I was once Director, but two silly mistakes in the book need correcting. ICRP did not introduce the concept of ALARA (As Low as Reasonably Achievable) in 1977, and never used it to avoid reducing dose limits, although it does decrease their importance. The idea of ALARA goes back at least to the 1950s, was firmly established by 1965 and was the subject of an ICRP report (Publication 23) in 1973.

As to the second error, Caufield visited

● This autumn, W.W. Norton is issuing paperback editions of the following books in Britain. *Cosmic Dawn: The Origins of Matter and Life* by Eric Chaisson. £6.50. (For review see *Nature* 336, 291; 1988.) *Longing for the Harmonies: Themes and Variations from Modern Physics* by Frank Wilczek and Betsy Devine. £7.95. (*Nature* 333, 220; 1988.) *Oasis in Space: Earth History from the Beginning* by Preston Cloud. £12.95. (*Nature* 331, 313; 1988.)

NRPB and knows that the Board is an advisory body. It could never have "directed that the dose limit for workers be immediately reduced from 5 rems per year to 1.5 rems per year" (p. 247). Indeed, it did not even recommend such a reduction. It did suggest that, *while the limits remain at their present level*, it would be prudent to adopt some time-averaged restriction so as to achieve an *average effective dose*

equivalent of 15 mSv per year.

This is a good book, one that deserves a wide audience. It does not tell the whole story, nor is it without bias. But then, books without bias make dull reading, which is not a charge that can be levelled at Catherine Caufield. □

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In the country

John S. Perry

The Weather Journals of a Rutland Squire: Thomas Barker of Lyndon Hall (1722–1809). Edited by John Kington. Rutland Record Society, Rutland County Museum, Catmos Street, Oakham LE15 6HW, UK: 1989. Pp.217. Pbk £16.50.

IN the journals of the Squire of Lyndon Hall, 1751 was a year "all on the extremes". His bees had been "so weaken'd by the cold and wet mid May which hindered their working for their numerous young ones, that without feeding them . . . several would have dyed". Oats were 12 shillings a quarter in the British Midlands "country" of Rutland. In other countries, the French vintage was bad; earthquakes troubled Finland; droughts beset Italy and Carolina. But in Rutland, 2.656 inches of rain were to be found in the Squire's cistern gauge, situated 7 ft 3 inches above the ground.

Employing no doubt the same scrupulously legible round hand of even the earliest of his journals, the Squire in later years communicated to the Royal Society's *Philosophical Transactions* not only the weather of Lyndon Hall, but also his calculations of comets' orbits and astronomical inferences from Eratosthenes and Ptolemy. To other audiences he presented his theological views on the benefits of baptism.

Thomas Barker, grandson of William Wiston, Newton's protégé and successor at Cambridge, emerges from the pages of this lovingly composed volume as a complex and vital man of his time, wedded to the soil of his comfortably self-contained "country" of Rutland, but intellectually linked with the larger worlds of politics, religion, science and, above all,

nature. His life spanned the most eventful years of the eighteenth century, but little of that turbulence intrudes into the voluminous journals that he began as a teenager. His lifelong passion was the ever-changing weather, together with its effects on his agrarian world. Thus, his meteorological journals contain not only notes of the conventional variables of pressure, temperature, wind and precipitation, but also detailed accounts of storms, droughts, floods, crop yields, pests — a rich, moving panorama of his century that has provided a treasure trove for students of past climate.

John Kington, a climatologist at the

from its members. But few of these records acquired the quality, continuity and wide dissemination of Barker's, which from 1771 were annually published in the *Philosophical Transactions*. Barker's reputation spread far beyond Rutland, for he was invited to contribute to an account of the severe winter of 1789–1790 being compiled by the Académie des Sciences of the unfortunate Louis XVI.

As Kington's account amply demonstrates, Thomas Barker was a pioneer in appreciating the importance of well-controlled, quantitative, continuous and extended observations in the study of nature. His journals show a keen awareness of the regularities of the British climate, and of the longer-term climatic fluctuations of his time. As a meteorologist of sorts, I stand in awe of Barker's insight and industry. But what sort of man was he? Some hints emerge from Kington's scholarship. A friend thought that marriage might relieve his "extreme Abstractedness & Speculativeness". At 61, he had a "streight belly", undertook a journey of 118 miles on horseback "without the least complaint or fatigue"

and ran foot races with his son Samuel.

One leaves Kington's charming monograph with lasting respect for this trail-blazing climatologist — and with a wistful longing to know more of Barker the man. Whilst waiting for a fuller biography, I try to close the gap of centuries by picturing him in today's Rutland, now politically homogenized with "countries" he would have termed foreign. I see him at Lyndon Hall busily puttering away with his PC, his ham radio, his collection of tropical fish. Of course, Lyndon Hall's acres would be festooned with automatic weather stations. On his bookshelves, beloved classics would be joined by Kafka and Kundera, and he

would no doubt struggle with enormous telephone bills and maintain lively correspondence equally with the *Times* and specialist journals. In the stables would stand his vintage Bugatti, next to his well-used Range Rover. This eighteenth-century man of the country would have been thoroughly at home as a twentieth-century man of science and the world. □

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Bar. Ther. Clouds Wind. Rain. May 1784. 225

26 35	24.59	63.4	600	W	1	W 2	chiefly cloudy morning
14 5	65	63.8	65.5	W	2	W 2	in the p.m. a few drops, sunny
26 45	87	60.2	51.5	W	2	var 1	in p.m. a great wind and very low in the night
14 0	58	61.3	64.4	W	1	W 1	clear or sunny calm and hot
22 25	84	59.4	53.4	W	1	SE 1	Wind morning var. high. S.W. W 4
14 5	79	62.3	67.4	00	0	0	in evening near it calm and hot
06 55	65	62.9	59.0	W	1	0	Wind S.W. 1
14 15	49	69.0	76.0	SW	1	W 2	in morning, clear, hot, windy for
24 62.5	44	65.5	56.4	WSW	1	SW 1	in evening much about the middle of the night
14 35	49	65.1	60.8	—	—	—	part lightning S.W. at night
26 20	52	62.0	55.3	—	—	—	Wind S.W. 1
14 45	46	65.7	71.0	W	1	SW 2	Sunny calm and pleasant
26 20	21	66.3	63.0	SE	2	SE 2	in morning and evening
14 30	17	69.4	73.8	—	—	—	Sunny and hot at night, calm
26 20	38	65.2	53.2	WSW	2	W 2	Wind S.W. 1
14 50	51	61.3	63.5	—	—	—	part of a thunder shower early in the morning

Weather report — an extract from the *Meteorological Journal* 1777–1789 for May 1784, showing daily observations, made by Thomas Barker, of pressure, temperature, clouds, wind, rain and weather.

University of East Anglia who studies eighteenth-century weather, has given us in this engaging little volume an ample selection of materials from the journals of Thomas Barker, together with brief but insightful sketches of the scientific and cultural worlds within which he lived, and the observational tools and methods he employed. Meteorology as a quantitative science was young. The barometer was less than a century old; the thermometer scarcely a half-century older. In 1723, when Barker was a toddler, the Royal Society began collecting weather journals

Does HIV cause AIDS?

"A dispute has broken out recently about the cause of AIDS. While the majority of virologists adhere to the common view that AIDS is caused by a virus that owes its name to this very belief, i.e., the Human Immunodeficiency Virus (HIV), a smaller number of heretics headed by the retrovirologist P. Duesberg, challenges this view. To express it in the words of the main opponents: "HIV causes AIDS!" (W. Blattner, R. C. Gallo and H. M. Temin) versus "HIV is a harmless, idle retrovirus that is not the cause of AIDS!" (P. Duesberg). In our time it has become quite rare that opposite positions are held so fiercely by highly respected scientists. There is public interest in a resolution of the controversy, since knowledge of the cause of disease is the basis of preventive measures." ■

Manfred Eigen contrasts these two views and discusses them critically in his article *The AIDS Debate* in the August issue of *Naturwissenschaften*. This monthly journal ranks among the most frequently cited in the natural sciences and is a journal of international importance. ■ If you are interested in the article or in more information about *Naturwissenschaften*, please write to: Springer-Verlag, 'Naturwissenschaften', P. O. Box 105280, D-6900 Heidelberg, FRG

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On the bench

Lisa Steiner

Antibodies: A Laboratory Manual. By Ed Harlow and David Lane. Cold Spring Harbor Laboratory Press: 1988. Pp.726. Spiral-bound \$50.

"It is only shallow people who do not judge by appearance" said Oscar Wilde. The mere look of *Antibodies* struck a positive chord, which puzzled me until I realized that it was due to the resemblance of the book to the familiar and indispensable *Molecular Cloning* (by Maniatis, Fritsch and Sambrook), also published by Cold Spring Harbor Laboratory Press. Indeed, the authors begin their preface with the comment that, inspired by the "cloning manual", they aim to put current immunochemical methodology into the hands of non-specialists. Happily, Harlow and Lane have succeeded admirably and this manual seems certain also to gain a place on the laboratory bench (too useful to remain on the bookshelf!) of a wide variety of biologists — even immunologists.

The volume opens with four introductory chapters summarizing key features of the immune response; these provide a succinct overview of the field and are very well written. The concepts of diversity, specificity, memory, clonal selection and tolerance are introduced with a minimum of fuss. The antibody molecule, its essential structural features that account for diversity and specificity, as well as the underlying genetic events, are economically surveyed. Antigen-antibody interactions are considered largely from the perspective gained from recent crystallographic analyses of complexes of monoclonal antibodies and protein antigens. The concepts of antibody affinity, avidity and multivalent binding, so important in applications, are also considered. Finally, the authors do a masterly job of discussing the essential features of antigen presentation and the role of B and T lymphocytes in the immune response, relating some of these basic principles to practical matters such as techniques of immunization. Each of the introductory chapters is accompanied by an up-to-date list of references.

The introduction is indeed a foretaste of good things to come. The scope of the rest of the book includes techniques of immunization and bleeding, monoclonal antibodies and hybridomas, protein labelling, immunoassays (mostly those using solid supports), immunoprecipitation of cellular antigens, immunoblotting and immunoaffinity purification. There are also four appendices containing much useful information: methods for running SDS gels, including a comparison of the sensitivities of autoradiography and fluorography, compilations of data about

proteins and amino acids, and a thoughtful selection of "general information".

The "oversimplifications and omissions" mentioned in the introductory chapters did not trouble me, with perhaps one exception: the lack of distinction between the terms antibody (a molecule that reacts specifically with a particular antigen) and immunoglobulin (essentially the family of all antibodies, regardless of specificity). Although the authors do state that they will not make this distinction, not everyone will read — or remember — this disavowal, and references to "pure antibodies" or "purification of antibodies" are frequently ambiguous. The problem is partly but imperfectly corrected by use of the term "specific antibody".

The methods are presented as easy-to-follow "1-2-3 protocols", and assume relatively little experience. Rationales for procedures are often presented, which helps to remove some of the mystique that has often been associated with immunological methodology. Although it is hazardous to evaluate a cookbook without getting into the kitchen, the descriptions of techniques seem mainly clear and reasonable, and there are only a few, generally minor, errors. Key references are given and there is an extensive list of them at the end of the volume (though it is not correlated with the various procedures). In some cases, more specific documentation would have been helpful. For example, Table 3.1 lists affinity values required for several common immunochemical techniques. Such data are not easy to obtain and I would have liked to have been able to look up the sources. In the section "Making Weak Antigens Strong", it would have been worthwhile to include examples of the use of antigen modification to enhance immunogenicity: only dinitrophenylation of actin is mentioned, and that without a reference.

Although acutely aware of the evolution in immunological technology, I am not sure I fully grasped its extent until I compared the contents of this manual with Kabat and Mayer's *Experimental Immunochimistry*, the techniques 'bible' for a previous generation of immunologists. Although *Antibodies* includes a chapter entitled "Immunoprecipitation", the quantitative precipitin reaction is not mentioned, nor will one find out how to do immunoelectrophoresis. And, unless I missed it, the word 'complement' does not appear. I venture to guess, however, that the usefulness of this volume will rival that of its distinguished predecessor. For current versions of more traditional methods, the reader must turn to other sources, and in the preface reference is made to some of them. □

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Interaction between peptide growth factors and homoeobox genes in the establishment of antero-posterior polarity in frog embryos

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The expression of the *Xenopus* homoeobox gene *xhox3* is an early response to mesoderm induction by peptide growth factors and the level of *xhox3* expression marks the antero-posterior character of the induced mesoderm. Different peptide growth factors specify different antero-posterior mesodermal cell fates as seen by the level of *xhox3* expression and the capacity to induce specific secondary neural/epidermal structures. These factors and homoeobox genes thus form part of the mechanism necessary for establishing antero-posterior polarity in the frog embryo.

POSITIONAL information for the antero-posterior axis of an amphibian embryo is first set out in the mesoderm¹ which in turn dictates antero-posterior differences in the overlying layer of ectoderm. Thus, an explanation of how the body plan of a frog tadpole is established depends in part on understanding how the embryonic mesoderm is formed and patterned.

Embryonic mesoderm is formed at the early blastula stage by an inductive interaction between endoderm and ectoderm² (Fig. 1). Recent studies show that this induction is likely to be caused by maternally encoded peptide growth factors (PGFs), including members of the fibroblast growth factor (FGF) and transforming growth factor- β (TGF- β) families³⁻⁷.

Understanding the spatial patterning of the mesoderm is not straightforward because the torus of mesoderm of the blastula undergoes an extensive deformation as it invaginates during gastrulation (Fig. 1). Studies on early patterning have shown that dorso-ventral polarity is initiated by a rotation of the egg's cytoplasm shortly after fertilization^{8,9}. Results from different experimental approaches have implicated the timing and extent of cell movements¹⁰, inductive interactions between cells^{11,12} and PGFs^{3,4,13} in establishing mesodermal fates but have not resolved certain questions, such as whether the antero-posterior and dorso-ventral mesodermal fates are specified by the same or entirely independent mechanisms.

The *Xenopus* homoeobox gene *xhox3* is one of the first genes to be transcribed in the early embryo and is expressed in a graded fashion along the antero-posterior axis in the axial mesoderm¹⁴ (Fig. 1g). Over-expression of *xhox3* prevents correct development of the anterior region where the gene is normally expressed at its lowest levels¹⁵. These findings have led us to suggest that *xhox3* is involved in interpreting positional information in the axial mesoderm along the antero-posterior axis. This raises the question of how the graded distribution of *xhox3* transcripts is first established.

Using the levels of *xhox3* expression as a mesodermal marker along the antero-posterior axis, we show here that different PGFs coordinately determine the level of *xhox3* messenger RNA and antero-posterior pattern in the developing embryo.

Xhox3 expression is induced by PGFs

At the early blastula stage, vegetal (endodermal) cells induce overlying animal (ectodermal) cells to change their fates to become mesoderm. Animal cap cells from a blastula can be induced *in vitro* to form mesoderm by combination with blastula vegetal cap cells (the natural inducers²) or by incubation with PGFs (refs 3, 4). Analysis of *xhox3* expression in isolated animal and vegetal caps and recombinates shows that *xhox3* is transcribed only in recombinates (Fig. 2a). In this case, mesoderm induction is verified by the expression of a gene for muscle-specific actin (ms-actin)¹⁶.

Incubating animal caps in either XTC-MIF, a *Xenopus* PGF-like mesoderm inducing factor of the TGF- β family^{3,17}, or basic fibroblast growth factor (bFGF)^{4,5}, results in the expression of *xhox3* mRNA (Fig. 2b). Notably, other factors such as platelet-derived growth factor, which is found in frog embryos¹⁸, or retinoic acid, do not produce this effect. As before, *xhox3* expression is observed only when mesoderm induction has taken place, as confirmed by the expression of ms-actin RNA. Interestingly, *xhox3* expression is also seen when mesoderm induction is triggered intracellularly by the polyoma middle T antigen (ref. 19). This viral oncogene is thought to mimic an endogenous component of the signal-transduction pathway used by PGFs in mesoderm induction. Together these data show that PGFs induce the expression of *xhox3* in animal cap cells that have been induced to form mesoderm.

Xhox3 expression and mesoderm induction

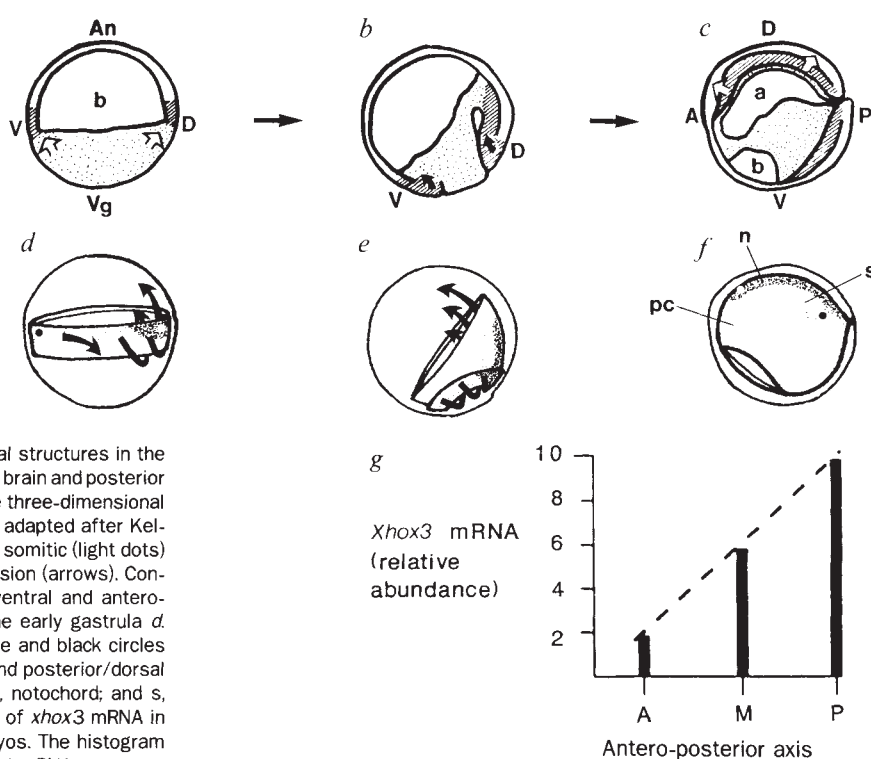
Xhox3 expression in XTC-MIF- or bFGF-induced mesoderm is easily detected by the early gastrula stage (Fig. 2c). Expression of *xhox3* in mesoderm occurs very early and is transient, in contrast to the relatively late and permanent expression of a tissue-specific mesodermal marker such as ms-actin (Fig. 2c). For both of these genes, expression in induced animal caps follows the normal schedule of expression in unmanipulated embryos.

In several organisms, proto-oncogenes²⁰⁻²² and genes that contain a homoeobox²³⁻²⁵ or zinc-finger²⁶ domain are expressed as an early response to a variety of inducing/activating agents. The expression of these genes, including *xhox3*, probably represents the activation of specific transcriptional programmes in response to a change in cell fate. Whereas *xhox3* expression is an early response to mesoderm induction, it is not sufficient on its own to cause mesodermal induction. The injection of synthetic *xhox3* mRNA can be used to direct ectopic expression of *xhox3* in animal caps, but this does not induce mesoderm (data not shown).

Mesodermal tissue induction by PGFs

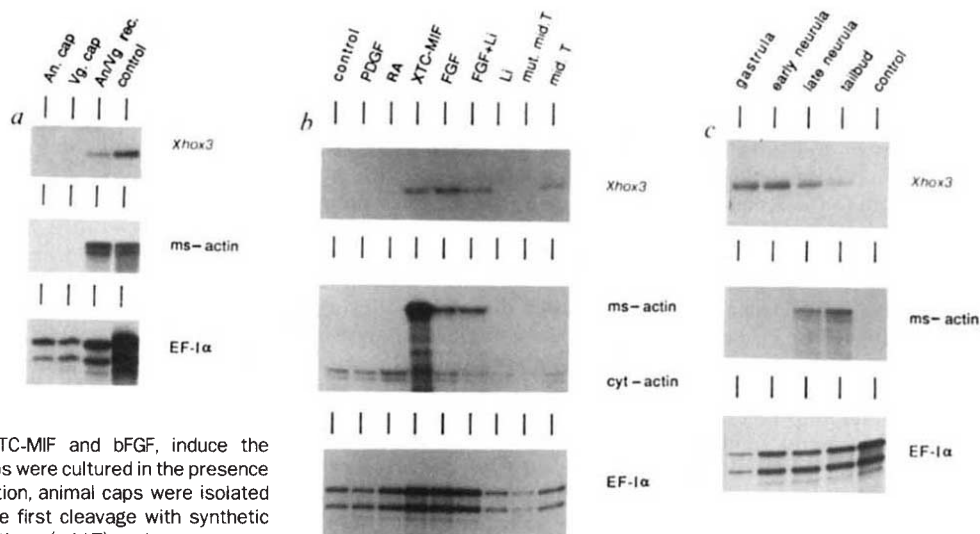
It has been suggested that FGF- and TGF- β -related growth factors are ventral and dorsal mesoderm inducers, respectively³⁻⁵. This notion is based primarily on two observations. First, bFGF induces mostly muscle, some blood and, rarely, notochord^{4,27}, whereas XTC-MIF induces a great deal of muscle and notochord^{3,17}. Thus, the mesodermal tissue induced by XTC-MIF is more dorsal than that induced by bFGF. Second,

FIG. 1 Drawings of *X. laevis* blastula and gastrula embryos showing inducing inductive and morphogenetic events. *a*, Mesoderm (hatched area) is formed in the blastula by an inductive interaction (open arrows) between endoderm (dotted area) and ectoderm (white area) along the animal (An)-vegetal (Vg) axis. The induced mesoderm forms a torus around the vegetal end of the blastocoel (b) as shown in *d*, *b*. Differences in the dorso-ventral axis established shortly after fertilization are evident in the different gastrulation movements (solid arrows) of dorsal (D) and ventral (V) mesoderm. Dorsal mesoderm invaginates first and migrates farther. *c*, At the end of gastrulation, the dorsal axial mesoderm has finished its invagination, creating the antero-posterior axis and forming the archenteron (a), the primitive gut. Note that the blastocoel is pushed ventrally during gastrulation. By this time, the axial mesoderm has induced neural structures in the overlying dorsal ectoderm; anterior (A) mesoderm induces brain and posterior (P) mesoderm induces spinal cord (open arrows). *d*–*f*, The three-dimensional aspects of mesodermal movements during gastrulation, adapted after Keller¹⁰. Note that prospective notochordal (heavy dots) and somitic (light dots) mesoderm is drastically rearranged by convergent extension (arrows). Consequently, the orthogonal relation between the dorso-ventral and antero-posterior axes of the late gastrula *f* is not found in the early gastrula *d*. As an example, the dorsal and ventral positions of white and black circles in the early gastrula are converted into anterior/dorsal and posterior/dorsal positions in *f*, the late gastrula. pc, Prechordal plate; n, notochord; and s, somitic mesoderm. *g*, Diagram of the graded expression of *xhox3* mRNA in the axial mesoderm of late gastrula-early neurula embryos. The histogram shows the relative abundance of *xhox3* mRNA assayed by RNase protection¹⁴. The highest value corresponds to the posterior third (P) and was equated arbitrarily to 1.0 and the values for middle (M) and anterior (A) parts



were set proportionately. *Xhox3* is expressed in the mesoderm of the early gastrula but its distribution in the prospective axes has not been determined.

FIG. 2 Expression of the homoeobox gene *xhox3* is an early response to mesoderm induction by peptide growth factors. *a*, *xhox3* mRNA expression in animal/vegetal recombinates (An/Vg rec). Animal and vegetal caps from dissected blastula were cultured in isolation or in combination and allowed to develop to the equivalent of the neurula stage. RNase-protection assays were performed for transcripts from the genes encoding *xhox3*, muscle-specific (ms) actin (a mesodermal marker), and elongation factor (EF)-1 α (ref. 47), a translation factor expressed in all embryonic cells which therefore serves as a control for RNA recovery. The control lane is RNA from neurula. *b*, Peptide growth factors that induce mesoderm, XTC-MIF and bFGF, induce the expression of *xhox3* RNA. Isolated animal caps were cultured in the presence of a variety of PGFs or morphogens. In addition, animal caps were isolated from embryos that had been injected before first cleavage with synthetic mRNA coding for the polyoma middle T antigen (mid.T) and a non-transforming mutant (NG59) of middle T (mut.mid.T) as control¹⁹. In all cases, RNA was isolated after the animal caps reached the equivalent of neurula stage and analysed by RNase protection, as above. Cytoskeletal actin (cyt-actin) was a control for RNA recovery. Untreated animal caps were included as negative controls. RA, retinoic acid. *c*, *Xhox3* expression is an early response to mesoderm induction by PGFs. Isolated animal caps were treated with XTC-MIF and allowed to develop for different times; RNA was isolated and assayed for the presence of *xhox3*, ms-actin and EF-1 α encoding mRNAs. Untreated caps were processed as negative control. The increase in the level of EF-1 α transcripts between the mid gastrula and early neurula stages is due to new transcription of this gene after the mid blastula transition⁴⁷. **METHODS.** *X. laevis* embryos were cultured in 0.1 $\times$ MMR (10 mM NaCl, 0.2 mM KCl, 0.1 mM MgSO₄·7H₂O, 0.2 mM CaCl₂·2H₂O, 0.5 mM HEPES, pH 7.6, 0.01 mM EDTA) and animal or vegetal caps were cut at the late blastula stage in the presence of streptomycin and penicillin on agarose dishes. Recombinates were made by joining the inner surfaces of animal



and vegetal caps and allowing them to fuse. Treatment of the isolated animal caps was as follows: recombinant human PDGF was used at a final concentration of 50 μ g ml⁻¹, bovine bFGF at 50 μ g ml⁻¹, partially purified frog XTC-MIF at a 1:50 dilution, and retinoic acid at 10⁻⁵ M. Lithium ion was applied at the early blastula stage by incubating whole embryos in 0.25 M lithium chloride for 8 min. This treatment reproducibly produced the development of anteriorized embryos which develop only heads³⁰. These embryos were rinsed in 0.1 $\times$ MMR and animal caps were cut at the blastula stage. Synthetic mRNAs were injected before first cleavage at ~4 ng per egg. RNA extraction and quantitative RNase protection assays using probes for all these genes are described elsewhere^{14,48}. RNA from ten, three and one-half animal cap equivalents was used for the *xhox3*, actin and EF-1 α assays, respectively. Exposure periods were for one week (*xhox3*), overnight (actin) and a few hours (EF-1 α). Only the relevant protected fragments from the autoradiographs are shown.

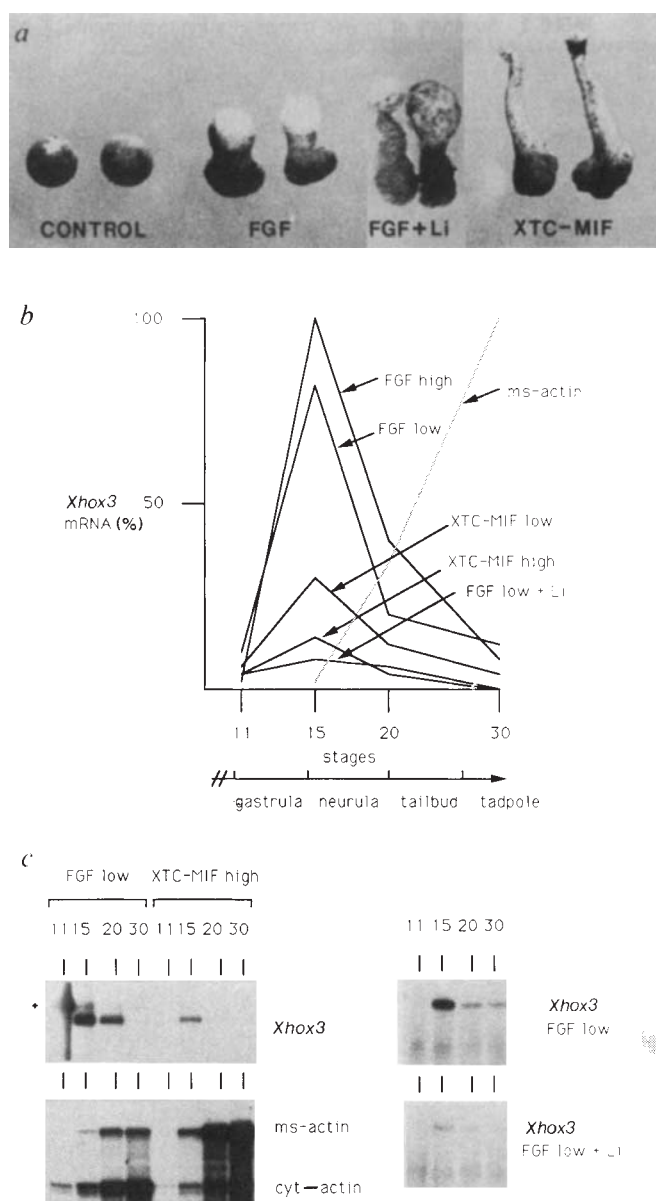


FIG. 3 Different peptide growth factors induce different levels of *xhox3* mRNA. **a**, Elongation of animal caps treated with bFGF at $50 \mu\text{g ml}^{-1}$, bFGF at $50 \mu\text{g ml}^{-1}$ plus lithium and XTC-MIF at 1:50 dilution. The controls are untreated animal caps. All caps were photographed at a time corresponding to the end of gastrulation (stage 13). **b**, Expression profile and quantitation of *xhox3* mRNA in caps treated with bFGF, bFGF plus lithium and XTC-MIF at different concentrations. Groups of 50 isolated animal caps were treated and time points collected at different stages. RNA was subsequently isolated and assayed by RNase protection for the presence of *xhox3*, actin and EF-1 α mRNAs as noted above. Only the results for the *xhox3* protections are shown in the graph after densitometry and normalization. In all cases, PGF-induced mesoderm expressed ms-actin as shown for one case only (bFGF at $50 \mu\text{g ml}^{-1}$ is shown, dotted line). FGF high, $200 \mu\text{g ml}^{-1}$; FGF low, $50 \mu\text{g ml}^{-1}$; XTC-MIF high, 1:50; XTC-MIF low, 1:200. Embryonic stage numbers refer to Nieuwkoop and Faber stages⁴⁹. **c**, Representative results from RNase protection assays. The expression profiles for *xhox3*, ms-actin and cyt-actin mRNA after induction bFGF at $50 \mu\text{g ml}^{-1}$ and XTC-MIF at 1:50 are shown on the left. XTC-MIF induces lower levels of *xhox3* mRNA even though it induces larger amounts of ms-actin. The cross notes a residual amount of undigested probe. The right panels show the expression profile of *xhox3* mRNA in caps treated with bFGF ($50 \mu\text{g ml}^{-1}$) or with bFGF ($50 \mu\text{g ml}^{-1}$) plus lithium. Both panels are part of the same gel and the kinetics of *xhox3* expression is the same; only the level of *xhox3* mRNA varies. Embryonic stages are shown at the top of each gel lane. Stages are as in **b**.

animal caps treated with XTC-MIF elongate more than those treated with bFGF (ref. 28) (Fig. 3a), mimicking the different movements of prospective dorsal and ventral mesodermal cells during gastrulation^{8,10}.

Whereas varying doses of XTC-MIF and bFGF clearly induce different dorso-ventral mesodermal tissues, at high doses, when both PGFs induce dorsal mesodermal tissues (muscle), the caps elongate differently (Fig. 3a). As described earlier (Fig. 1), differences in elongation cannot be easily attributed to either dorso-ventral or antero-posterior differences in fate^{8,10}, which raises the possibility that XTC-MIF and bFGF affect both fates. For example, it is possible that these PGFs induce a state that enables mesodermal cells to acquire a specific dorso-ventral and antero-posterior fate during gastrulation.

Xhox3 mRNA induction by PGFs

To test for the induction of dorsal mesoderm with different antero-posterior character, we probed for the level of *xhox3* mRNA at the early neurula stage, when *xhox3* expression shows clear antero-posterior differences in normal embryos¹⁴. In these experiments, both XTC-MIF and bFGF were used at concentrations that induce dorsal mesoderm in animal caps, as judged by the expression of ms-actin. Figure 3b shows that bFGF induces higher levels of *xhox3* mRNA than does XTC-MIF. At any concentration of PGF sufficient to induce muscle, bFGF produces higher levels of *xhox3* mRNA than does XTC-MIF. Whereas the amount of *xhox3* RNA induced in XTC-MIF treated caps is lower than in those treated with bFGF, the expression of ms-actin is greater in the former (Fig. 3c). This indicates that the low amount of *xhox3* RNA in XTC-MIF treated cells is not due to the induction of fewer cells to become muscle as compared with treatment with bFGF.

The five- to tenfold difference observed in the amount of *xhox3* mRNA induced by XTC-MIF and bFGF is similar to the endogenous difference in *xhox3* mRNA levels between anterior and posterior mesoderm of the early neurula embryo¹⁴ (Figs 1, 3). As high and low levels of *xhox3* expression are correlated with posterior and anterior determination, respectively^{14,15}, this suggests that high doses of bFGF induce dorsal/posterior mesoderm, whereas high doses of XTC-MIF induce dorsal/anterior mesoderm. Consistent with this, we note that increasing the dose of XTC-MIF does not increase, but rather decreases the amount of *xhox3* mRNA that is induced (Fig. 3b). Perhaps the highest XTC-MIF doses induce the most anterior mesoderm which, in turn, has the lowest level of *xhox3* mRNA (see Fig. 1).

Antero-posterior character of mesoderm

Treatment of blastula stage embryos with high doses of lithium gives the mesoderm a more anterior and dorsal character, whereas treatment with moderate doses results only in anteriorized mesoderm. In the latter case, the resulting embryos develop only head structures with normal dorso-ventral polarity^{29,30}. Treatment with lithium alone has no effect on the development of isolated animal caps, but treatment with lithium plus bFGF results in more extensive elongation as compared with animal caps treated with bFGF alone³¹ (Fig. 3a). This suggests the possibility that bFGF induces posterior mesoderm whereas bFGF plus lithium induces anterior mesoderm. This allows further testing of the correlation between *xhox3* levels and the antero-posterior character of the induced mesoderm.

The level of *xhox3* expression in bFGF-induced mesoderm anteriorized by lithium is lower than that observed in animal caps treated with bFGF alone (Fig. 3b, d). In both cases, ms-actin is expressed at high levels (data not shown). Notably, the difference in the levels of *xhox3* mRNA in these two cases is again about five- to tenfold (Fig. 3b), as with the antero-posterior difference in normal embryo mesoderm¹⁴. These observations strengthen the correlation between *xhox3* levels and antero-posterior mesodermal cell fates.

Testing for antero-posterior character

The experiments described above demonstrate that there is a good correlation between induction by a particular PGF and the level of *xhox3* RNA, but they do not directly test whether the mesoderm in PGF-induced animal caps is anterior or posterior. We have, therefore, directly tested the antero-posterior character of PGF-induced mesoderm by assaying its ability to induce specific antero-posterior secondary neural/epidermal structures. This is possible because the distinct epidermal and neural structures found along the axis are a direct reflection of antero-posterior differences in the underlying dorsal mesoderm which induces those ectodermal structures^{1,11,32}. Dorsal mesoderm from an early gastrula (an organizer) can induce a secondary axis when grafted into the ventral marginal zone of a host embryo^{1,33,34} (Fig. 4a). In a different assay, an organizer implanted into the blastocoel of a host embryo will induce anterior (head) structures (Fig. 4b) and sometimes a secondary axis. This latter assay was used to test the antero-posterior character of dorsal mesoderm from an early neurula salamander embryo³². It was found that anterior and posterior mesoderm induce the corresponding neural/epidermal structures after implantation, for example anterior mesoderm induces brain and eyes, whereas posterior mesoderm induces spinal cord and dorsal fin.

Implantation of animal cap pieces previously induced with high doses of XTC-MIF causes a secondary induction of anterior (head) structures as seen externally (Fig. 4c) and assayed histologically (Fig. 5b, c). Animal caps treated with bFGF induce the formation of posterior (tail) structures (Figs 4d, 5d); anterior structures are never observed. As a control, untreated animal cap material shows no inductive capacity in this assay (Fig. 4e). Thus, different PGFs induce mesoderm with different antero-

TABLE 1 Summary of *in vivo* transplantation experiments

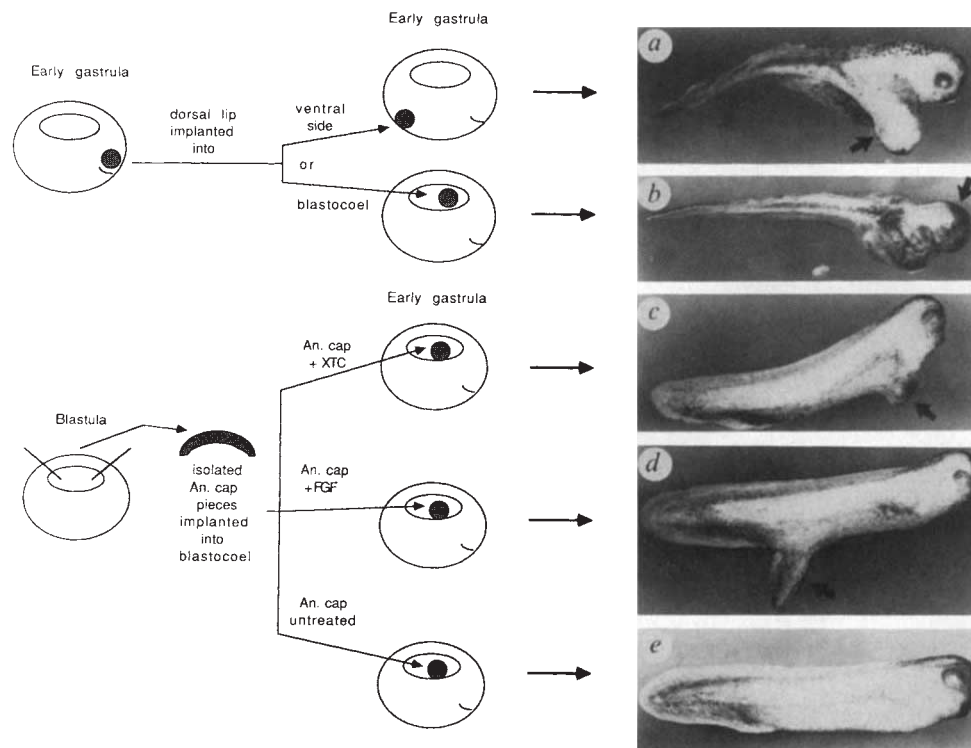
Animal cap treatment	Induced secondary structures				
	Head	Trunk	Tail	Normal	Total
XTC-MIF 1:50	30 (52%)	7 (12%)	10 (17%)	11 (19%)	58
XTC-MIF 1:100	2 (7%)	6 (21%)	15 (54%)	5 (18%)	28
XTC-MIF 1:500	0 (0%)	0 (0%)	2 (12%)	14 (88%)	16
bFGF 50 ng ml ⁻¹	0 (0%)	6 (10%)	38 (63%)	16 (27%)	60
bFGF 200 ng ml ⁻¹	0 (0%)	2 (7%)	16 (55%)	11 (38%)	29
Untreated	0 (0%)	0 (0%)	1 (2%)	49 (98%)	50

Animal caps treated with different doses of XTC-MIF or bFGF were used in transplantation experiments as described in Fig. 4. The control was untreated animal caps transplanted to the blastocoel of early gastrula. All anterior structures such as cement glands and eyes were scored as head, most tails contained obvious caudal fins. In only a few cases were the heads well proportioned. The capacity of XTC-MIF to induce anterior (head) structures is progressively lost as the dose of the PGF is lowered: compare the XTC-MIF 1:50 to XTC-MIF 1:500. Results from 10 independent experiments are shown.

posterior character as judged by the level of *xhox3* expression in animal cap cells and also by the character of the secondary neural/epidermal structures that are induced by this mesoderm when implanted.

Whereas high concentrations of XTC-MIF produce mesoderm that can induce anterior structures (Fig. 4), lower concentrations result in the induction of posterior structures similar to those induced by bFGF (Table 1). This indicates the existence of a graded response to the dose of XTC-MIF, as well as a graded distribution of XTC-MIF or a similar molecule along the antero-posterior axis. We note that secondary head

FIG. 4 Peptide growth factors induce mesoderm with different antero-posterior character as seen by the ability to induce different secondary neural/epidermal structures. The positional value of mesoderm is tested by grafting *a* or implantation *b-e* of mesodermal pieces into gastrulating host embryos. *a*, When the dorsal lip of an early gastrula (an organizer) is grafted to the ventral marginal zone of a host gastrula, an entire second body axis is induced *b*, When the same mesodermal region is implanted into the blastocoel, it is pushed ventrally during gastrulation and induces primarily head structures and sometimes whole secondary axes. Animal caps induced by XTC-MIF or bFGF were implanted into the blastocoel of sibling embryos at the early gastrula stage. Animal caps induced by XTC-MIF at high doses (1:50) can induce head structures *c*, whereas bFGF never induces head structures and only induces tail and trunk structures *d* even at high doses (200 μ g ml⁻¹). *e*, Untreated animal caps have no effect following implantation as ectoderm is incapable of neural induction. Arrows show the secondary structures: in *a*, a whole axis; *b*, a head; *c*, a head with cement gland and optic cup, and in *d*, a tail with dorsal fin.



METHODS. Transplantation experiments were as described previously^{32,33}. Animal caps were isolated at the early blastula stage and incubated with the appropriate growth factors in 1×MMR with antibiotics. At a stage equivalent to that of the early- to mid-gastrula stage, the ectodermal layer was peeled off and the induced mesoderm tissue was implanted into host early gastrula stage embryos through a small hole in the blastocoel roof.

The size of the implants was equivalent to about half the size of a single animal cap. Host embryos healed within minutes and were allowed to develop in 0.1×MMR with the animal pole facing up. Dorsal marginal zones (organizers) were dissected from early (stage 10) gastrula and either implanted into the blastocoel or grafted to the ventral side of a sibling embryo after the extirpation of some ventral marginal zone cells.

and tail structures are always found at the host's anterior and posterior ends, respectively (see Figs 4, 5a). This could reflect antero-posterior differences in cell surface properties.

A given amount of *xhox3* mRNA is apparently necessary, but not sufficient, to specify antero-posterior position in mesoderm. For example, over expression of *xhox3* mRNA in the anterior end of normal embryos does not result in the production of posterior structures at the anterior end, but rather the failure to form normal anterior structures¹⁵. Also, even though the level of *xhox3* RNA can be lowered in bFGF-treated animal caps by treatment with lithium (Fig. 3), this anteriorized mesoderm does not acquire the ability to induce head structures in transplantation assays (data not shown). Thus, whereas the amount of *xhox3* RNA marks the positional value of mesoderm, changing *xhox3* RNA levels is not sufficient to change its antero-posterior character.

Mesoderm as a Spemann organizer

Grafting XTC-MIF induced animal cap mesoderm into the ventral marginal zone of a host embryo results in the formation of a secondary axis³⁵. We find that when high concentrations of XTC-MIF are used to induce mesoderm, secondary axes are also observed following transplantation of the induced mesoderm into the blastocoel of a host (Fig. 5). This is the same result as that observed when a full organizer from the dorsal blastopore lip of an early gastrula is transplanted into the blastocoel or ventral marginal zone of a host^{11,33}. Similarly, mesoderm induced by high concentrations of bFGF induces trunk structures (Fig. 4d, 5d). This corresponds to the results obtained following transplantation of a trunk organizer taken from the dorsal lip of a mid to late gastrula^{1,36}.

These experiments show that implanted animal caps treated with high doses of PGFs can redirect host ventral mesodermal cells to become dorsal with an antero-posterior character deter-

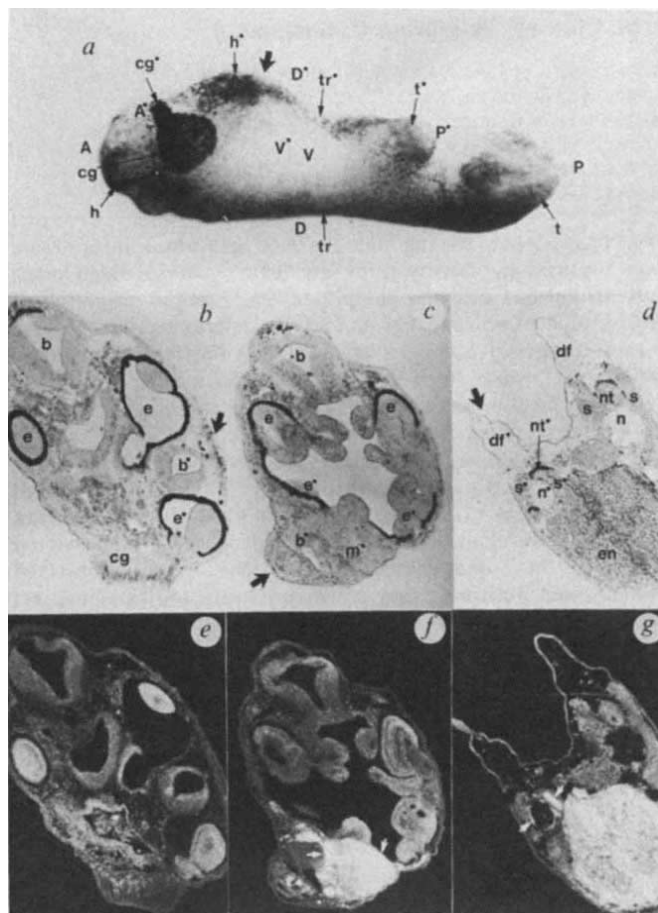
mined by the implant. Labelling the implant with a lineage tracer shows that most of the induced secondary mesodermal and neural structures are derived from host, not grafted tissue (Fig. 5e-g). Thus, treatment with PGFs not only induces mesoderm in animal cap cells, it endows this mesoderm with the properties of a Spemann's organizer, including dorsalization and neural induction^{1,11,33}.

Discussion

Endogenous PGFs of the FGF and TGF- β families probably induce embryonic mesoderm during early development^{3-7,37,38}. This newly induced mesoderm must attain regional differences which lead to axial patterning and the development of different tissues such as notochord, muscle and blood. Our results with the homoeobox gene *xhox3* lead us to propose that PGFs not only act as morphogens that pattern the dorso-ventral axis, but also the antero-posterior axis. We note that other PGFs such as TGF- β 1 are unable to induce mesoderm on their own, but can alter the response of animal cap cells to FGF^{5,6}. Thus, there may also be several PGFs in the embryo that act as patterning morphogens, but not mesoderm inducers.

The establishment of antero-posterior polarity requires a system of positional information³⁹ that specifies different fates for mesodermal cells according to their position at the end of gastrulation⁸. The system probably includes a diffusible graded signal⁴⁰ to account for the observed graded axial deficiencies in embryos obtained after perturbation by ultraviolet irradiation^{41,42}, gastrulation arrest⁹ or *xhox3* over-expression¹⁵. Our results indicate that this graded signal is likely to be one or more PGFs, as varying the dose of PGF used to induce mesoderm results in a graded response both in the amount of *xhox3* mRNA induced and in the antero-posterior character of the mesoderm (Fig. 3 and Table 1). In fact, the graded distribution of *xhox3* transcripts observed in the axial mesoderm of

FIG. 5 Mesoderm induced in animal cap cells by peptide growth factors has the properties of a Spemann organizer. *a*, Animal cap cells treated with XTC-MIF (1:25–1:50) was implanted into the blastocoel of an early gastrula as shown in Fig. 4c. Ventral mesoderm and ectoderm of the host is induced to form a whole secondary axis including anterior, middle and posterior neural/epidermal structures. A ventral view of the host embryo shows the secondary axis (thick arrow) with secondary cement gland (cg), head (h), trunk (tr) and tail (t). All secondary structures are denoted by a dot superscript. Because the blastocoel containing the implanted mesoderm is pushed ventrally during gastrulation, the secondary axis usually appears ventral to the host axis and the two embryonic axes are joined at their ventral sides. Anterior is to the left. *b–g*, Histological analysis of secondary structures in three separate animals. These examples confirm the external identification of secondary anterior and posterior structures as shown in Fig. 4 and described in Table 1. Animal caps isolated from embryos injected with the lineage tracer FLDx (ref. 34) were induced to form mesoderm by XTC-MIF (1:50; *b, c*) or bFGF (100 μ g ml⁻¹; *d*). As in *a*, PGF-induced animal cap material was implanted into the blastocoel of an unlabelled host embryo. After sectioning the whole animal, photographs of selected sections were taken under bright field (*b–d*) or fluorescent illumination (*e–g*). Sections in panels *b* and *c* demonstrate that the induced secondary heads contain well defined brain (*b*) and eyes (*e*) as well as mesodermal (*m*) tissues. Panel *d* shows a second dorsal fin (*df*), neural tube (*nt*), somites (*s*) and notochord (*n*) induced by an animal cap treated with bFGF before implantation. Thick arrows point to the midline of the secondary axes. *e–g*, The cells derived from the FLDx-labelled implanted mesoderm are identified by their fluorescence (white arrows). Note that in *e* all secondary structures are host derived whereas in *f* there is a mixture of host and implant material in the secondary axis. In *e*, the FLDx-labelled descendants of the implanted cells are found in other sections (not shown). In *g*, all secondary structures are host derived with the exception of part of the somites and notochord. The bright signal in the endoderm (*en*) in *g* is due to autofluorescence of the yolk.



early embryos may be a reflection of the graded distribution of PGFs. In addition to effects on the expression of *xhox3*, PGF gradients may regulate the mesodermal and neural expression of other frog homoeobox genes including *xlhbox6*, (ref. 43) *xlhbox1* (ref. 44, 45) and *xhox36* (ref. 46). Thus, gradients of

PGFs could set positional information and polarity along the antero-posterior axis in the responding axial tissues by regulating the level of homoeobox gene expression. If this is the case, more needs to be learned about where endogenous PGFs are found and when (before, during or after gastrulation) they act. □

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LETTERS TO NATURE

Diagnostic properties of moving gravitational lenses

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GRAVITATIONAL lensing has provided important information about the mass and density of the intervening galaxies which image more distant quasars, and has placed limits on the properties of massive objects which might perturb the microwave background. In most studies it has been assumed that the lensing object is stationary^{1–3}; some work has been done on moving lenses, but these have mostly been concerned with the effects of motion on the light curve, particularly as caustics sweep past the observer^{4,5}, on the redshift pattern and surface brightness of a moving background^{6–8}, or as a cause of microlensing^{9,10}. Here we point out several properties of moving lenses that do not appear to have been noted before: moving lenses always produce two-dimensional velocities; they impose specific relations between observed velocities and positions; they magnify relative source velocities; they convert linear into rotary motion; and they convert radial into transverse velocity. The mass and distance of a moving lens can be separately determined. We point out some useful astronomical diagnostic properties of moving lenses, for which the Very-Long-Baseline Array under construction will be particularly useful.

To derive new physical properties of moving lenses in a simple way we consider point-mass gravitational lenses and use the geometry and notation of ref. 11. The gravitational lens of mass M is characterized by a strength parameter

$$\mu = \frac{4GM}{c^2} \frac{l_{OD}l_{DS}}{l_{OS}} = \theta_0^2 l_{OD}^2 \quad (1)$$

where l_{OD} , l_{DS} and l_{OS} are, respectively, the observer–deflector, deflector–source and observer–source distances, and θ_0 is the angular radius of the cone of inversion given by

$$\theta_0 = 2.9 \left(\frac{M}{M_\odot} \right)^{1/2} \left(\frac{l_{DS}}{l_{OD}l_{OS}} \right)^{1/2} \text{ mas} \quad (2)$$

with distances measured in kpc. If we project the source and image positions onto the deflector plane, then the distances of the source and image positions from the deflector, denoted by r and r_1 respectively, are related by

$$r_1 = r + \frac{\mu}{r_1} r_1 \quad (3)$$

Consider a system in which the lens and the source move relative to one another. In the absence of the lens, the source would seem to move with coordinates $x(t)$, $y(t)$. To a first approximation, for short times, the source motion will be linear and we can choose the cartesian coordinate system such that $x = vt$ and $y = a = \text{constant}$. Any more complicated lens or image can be built up by combining such fundamental solutions. Our simple example contains the essential physical properties of image motion and can be visualized easily.

The velocity components of images in the x_1 and y_1 direction, follow from equation (3):

$$V_{x1} = \frac{v}{2} \pm \frac{v}{2} \left(1 + \frac{4\mu}{a^2 + v^2 t^2} \right)^{1/2} \mp \frac{vt}{2} \frac{4\mu v t^2}{(a^2 + v^2 t^2)^2} \left(1 + \frac{4\mu}{a^2 + v^2 t^2} \right)^{-1/2} \quad (4)$$

and

$$V_{y1} = \mp \frac{2a\mu v t}{(a^2 + v^2 t^2)^2} \left(1 + \frac{4\mu}{a^2 + v^2 t^2} \right)^{-1/2} \quad (5)$$

A striking feature of moving lenses is their ability to alter the source velocities significantly. Even though the source has a

constant velocity relative to the lens, the image will seem to accelerate. Although the source velocity can be one-dimensional in a suitably rotated coordinate frame for short times, lensed velocities are two-dimensional for any coordinate rotation. From equations 3-5, there is also an important relation at any epoch between the velocities (V_{x1} , V_{y1}) and the image coordinates (x_1 , y_1):

$$V_{x1} = \frac{x_1}{t} + V_{y1} \frac{x_1}{y_1} \quad (6)$$

Furthermore, the two components of the image velocities yield the total squared transverse image velocity as a function of position

$$V_1^2 = V_{x1}^2 + V_{y1}^2 = \frac{x_1^2}{t^2} \left[1 - \frac{2x_1}{\mu} \frac{(x_1 - vt)^2}{(x_1 - vt/2)^2} + \frac{x_1^2 + y_1^2}{\mu^2} \frac{(x_1 - vt)^4}{(x_1 - vt/2)^2} \right] \quad (7)$$

The right-hand side of equation (7) reduces to v^2 in the limit $\mu \rightarrow 0$. The relations given by equations (6) and (7) are a signature of a moving gravitational point lens. More complicated lenses will have analogous velocity-position signatures, appropriate for more specific models.

The ability of the moving lens to magnify source velocities appreciably, especially when source and lens are closely aligned along the line of sight, is apparent from equations (4) and (5), rewritten as

$$\frac{V_{x1}}{v} = \frac{1}{2} [1 \pm (1 + 4\eta s)^{1/2} \mp 4\eta s(1-s)(1 + 4\eta s)^{-1/2}] \quad (8)$$

$$\frac{V_{y1}}{v} = -2\eta s^{3/2}(1-s)^{1/2}(1 + 4\eta s)^{-1/2} \quad (9)$$

where $\eta = \mu/a^2$ and $s = 1/(1 + \xi^2)$ with $\xi = vt/a$, considering v as constant for short periods.

Clearly V_{x1}/v can become arbitrarily large for sufficiently large values of η and small ξ . Figure 1 shows solid and dotted curves corresponding to V_{x1}/v and V_{y1}/v as functions of ξ , for a sample of values of $\eta = 1, 10, 10^2$ and 10^3 . The x and y components of the source velocities can be magnified by a

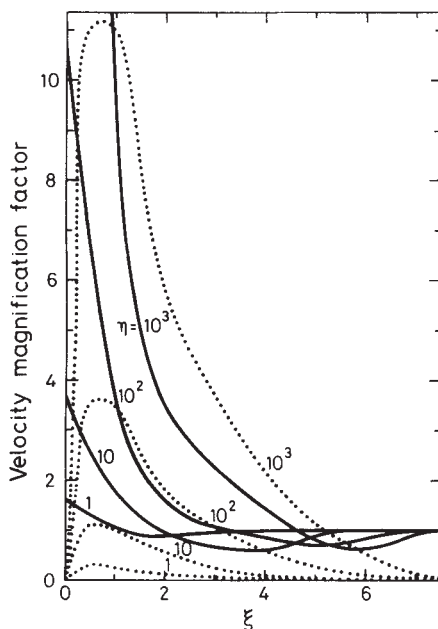


FIG. 1 The velocity magnification factors V_{x1}/v (solid lines) and V_{y1}/v (dotted lines) are shown as functions of $\xi = vt/a$ for values of $\eta = 1, 10, 10^2$ and 10^3 .

substantial factor (≥ 5) for values of $\eta \gg 10$ in the region $0 \leq \xi \leq 1$. Thus it is conceivable that the source velocities could be magnified by an order of magnitude or more, as long as the position of the source is projected well within the cone of inversion. Part of the image may then exhibit an apparent superluminal motion.

A second effect of moving lenses is to convert linear into rotary motion, as velocities are magnified by different amounts in different directions. The angular change resulting from translational motion is $\Delta\theta \approx 20vt/l_{OD}$ mas, where v is units of 100 km s^{-1} , t is in yr and l_{OD} is kpc (for $4\mu(a^2 + v^2t^2)^{-1} \ll 1$), which is measurable with very-long-baseline interferometry (VLBI) if the lens is in our galaxy. This is a conservative example because $\Delta\theta$ is magnified considerably when $\mu \gg a^2 \gg v^2t^2$.

The simplest example of the rotary phenomenon is illustrated by linear source motion for which the image trajectory in polar coordinates is

$$r_1 = \frac{a}{2} \left(1 + \frac{1}{\tan^2 \theta_1} \right)^{1/2} + \frac{a}{2} \left(1 + \frac{1}{\tan^2 \theta_1} + \frac{4\mu}{a^2} \right)^{1/2} \quad (10)$$

with

$$\theta_1 = \tan^{-1} \left(\frac{a}{vt} \right)$$

For $\mu = 0$ only would the image of the source motion be undistorted. This provides a means of estimating μ by observation if the source moves linearly with constant velocity, at least for short times.

The rotation of more general source motions and of extended sources is illustrated in Fig. 2, which shows the images as the source moves along a straight line (grey solid or dotted areas). The corresponding images indicated by black filled and dotted lines give the impression of a rotary motion as the source moves from left to right relative to the gravitational lens. The impression of rotation is enhanced as the moving dark image beyond the cone of inversion seems to merge with the moving dark image within the cone of inversion. An observer unaware of the source motion is apt to connect, incorrectly, these two images and to interpret the result as a net rotary motion.

A consequence of rotary motion is that the conversion of

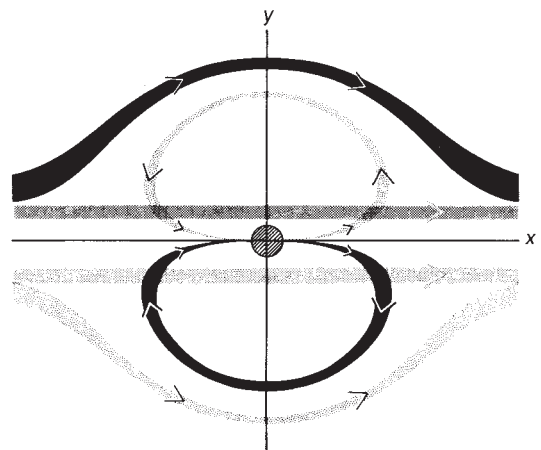


FIG. 2 Two straight line contours (solid and dotted) show the source motion parallel to the x -axis in the direction of the arrows. The corresponding external and internal images are indicated by dark and dotted contours; the hatched circle denotes the central deflector. The images marked by the arrows seem to give the impression of a rotary motion, as the source moves from left to right apparently merging the dark image outside the cone of inversion with the moving dark image within it.

uniform linear source motion is accompanied by an apparent acceleration. Some superluminal sources (for example, 3C345) seem to show accelerating components on VLBI scales¹², and it is conceivable that some observed superluminal velocities and accelerations could arise from lenses moving relative to the background components of a radio source.

A third feature of moving lenses is their ability to convert the radial component of a source's motion relative to the lens into a transverse component of image motion. This follows from equations (1) and (3) by letting μ vary as the deflector-source distance changes along the line of sight. From equation (1) we obtain

$$\dot{\mu} = \frac{4GM}{c^2} \left(\frac{l_{OD}}{l_{OS}} \right)^2 \dot{l}_{OS} \quad (11)$$

and the velocity components given by equations (4) and (5), derived with a constant μ , are modified to

$$V_{x1} = V_{x1} (\mu = \text{constant}) \\ \pm 5 \times 10^5 (M/M_\odot) \left\{ \frac{l_{OD}}{l_{OS}} \right\}^2 v_r \frac{\xi s}{a(1+4\eta s)^{1/2}} \quad (12)$$

and

$$V_{y1} = V_{y1} (\mu = \text{constant}) \\ \pm 5 \times 10^5 (M/M_\odot) \left\{ \frac{l_{OD}}{l_{OS}} \right\}^2 v_r \frac{s}{a(1+4\eta s)^{1/2}} \quad (13)$$

Here the relative radial component of source velocity is $v_r = \dot{l}_{OS}$. This term adds to the transverse components V_{x1} and V_{y1} of the image velocities, and V_{y1} ($\mu \neq \text{constant}$) can become significant relative to V_{x1} ($\mu \neq \text{constant}$) if ξ is small and η is large, because $s/[a(1+4\eta s)^{1/2}] \approx \mu^{-1/2}$. In general this will be a small correction to V_{x1} or V_{y1} which is unlikely to be observable with present techniques, although conversion of radial into transverse motion is a novel astrophysical effect.

Observed image motions can determine the distance and mass of the lens, provided the source is much farther away than the lens, or if there is independent knowledge of the observer-source distance. It is possible, in principle, to determine the source position from the positions of the two images ($x_1^+(t)$, $y_1^+(t)$), ($x_1^-(t)$, $y_1^-(t)$) produced by a lens

$$x = vt = x_1^+(t) + x_1^-(t) \quad (14)$$

$$y = a = y_1^+(t) + y_1^-(t) \quad (15)$$

and hence obtain μ from the observed velocities or corresponding image positions. Having derived μ and measured θ_0 from the observed image configuration, we can infer the value of l_{OD} from equation (1) and of M from equation (2) if $l_{OD} \ll l_{OS}$ or if l_{OS} is known independently.

The quantities η , v and s can be related directly to observations. If the source lies within the cone of inversion given by the angular radius θ_0 , then $\eta = \mu/\alpha^2$ is just the total observed intensity gain of the image¹. Therefore a consistency check is provided by measuring a and μ from the positions and velocities of the two images and measuring η from the relative intensities at different epochs. Furthermore, velocity observations from four epochs can provide an independent determination of v , μ and η .

These ideas can be applied in a number of specific cases. Although the close alignments required for lensing generally have a very low *a priori* probability, they do occur. Lensed quasars, quasars behind the nucleus of a nearby galaxy and galactic stars in front of extragalactic radio sources¹³ have all been discovered, usually by accident.

An interesting application is to the possible presence of a massive black hole, of a few million solar masses, in our galaxy's nucleus, for which there is significant, but not conclusive,

evidence.^{14,15} One way to infer its existence and properties would be to examine its influence on background sources such as radio clouds, jets and maser regions lying on the far side of the nucleus from us. Gravitational lensing phenomenon such as arcs or ring-like morphology at radio and infrared wavelengths would need to be confirmed by the velocity information we have discussed, because the complex structure of a galactic nucleus could also be due to phenomena unrelated to lensing.

For a black hole of mass M in the galactic nucleus, at a distance $l_{OD} = 10$ kpc, the typical angular image scale is, from equation (2):

$$\theta_0 = \left(\frac{M}{10^6 M_\odot} \right)^{1/2} \left(\frac{l_{DS}}{l_{OS}} \right)^{1/2} \text{ arcsec} \\ = 5 \times 10^{-2} \left(\frac{M}{10^6 M_\odot} \right) \left(1 - \frac{l_{OD}}{l_{OS}} \right)^{1/2} \text{ pc} \quad (16)$$

For a significant effect, the last factor would typically be ~ 0.5 . Maps of the galactic centre in the infrared¹⁶ and at 21 cm (ref. 17) show complex structure on scales ≥ 10 arcsec but, for these to be lensed images, the black-hole mass would have to exceed $10^8 M_\odot$ for which there is no corroborating dynamical evidence. It is more probable that lens images will appear on much smaller scales (≤ 1 arcsec) with radio and infrared interferometry. However, the necessary extensive multi-epoch VLBI and infrared mapping of our galactic nucleus on this scale has not yet been done.

The corresponding timescale for variations arising from the source motion with relative velocity v is

$$\tau = \frac{\theta_0 l_{OD}}{v} \\ = 500 \left(\frac{M}{10^6 M_\odot} \right)^{1/2} \left(\frac{l_{DS}}{l_{OS}} \right)^{1/2} \left(\frac{v}{100 \text{ km s}^{-1}} \right)^{-1} \text{ yr} \quad (17)$$

Image variations with timescales less than several years, and observable with resolution ≥ 0.5 mas, include hot spots in relativistic jets, black-hole accretion-disk flares, supernova and related phenomena with relativistic speeds, positioned well beyond the nucleus. Moreover, phenomena such as colliding stars or pulsars (which would produce a pulsating ring if perfectly aligned) with intrinsically short timescales, can occur in the nucleus. On the other hand, a slowly moving source imaged by a low-mass black hole or microlensed by a star in the nucleus can appear to vary rapidly. As our line of sight through the galactic nucleus passes roughly through the galactic plane it may fortuitously intersect a suitable source in a spiral arm, even though the *a priori* probability is very small.

A second illustration is maser clouds, which radiate over a small bandwidth. Narrow band VLBI can measure the relative positions of features in a narrow radial-velocity range very accurately¹⁸. For example, a source with velocity 1 km s^{-1} at a distance of 1 kpc would move an angular distance ~ 0.14 mas during a year, and this will be detectable. The distance l_{OS} to maser clouds such as Orion can be determined to $\sim 20\%$ accuracy from the proper motion of the many sources in the cloud¹⁸. Thus, if an individual moving source in a maser complex is lensed by a foreground star in our galaxy, or by a black hole in the galactic nucleus, the resulting velocity field of its images can be analysed to infer the mass and distance of the lens.

Extragalactic imaging provides a third illustration. It is widely thought that the engine powering extragalactic radio sources is a black hole with an accretion disk. There is also evidence for asymmetric nuclear radio jets which may exert a net thrust on the source causing it to behave like a massive rocket¹⁹. The resulting motion of the black hole may be inferred from the action of the moving lens on a suitably aligned background. Thus, if we view a source (a supernova or a hot spot in a radio jet, for example) that is behind an extragalactic object containing

a black hole of mass M , then the timescale for structural changes of the image of a background source is of order

$$\tau = \frac{\theta_0 l_{OD}}{v}$$

$$\approx (10,000) \left(\frac{M}{10^8 M_\odot} \right)^{1/2} \left(\frac{l_{DS}}{l_{OS}} \right)^{1/2} \left(\frac{10^4 \text{ km s}^{-1}}{v} \right) \text{ yr}$$

$$\approx 15 \text{ yr} \quad (18)$$

if $l_{DS} = 1 \text{ kpc}$, $l_{OS} = 500 \text{ Mpc}$, $M = 10^8 M_\odot$ and $v = 10^4 \text{ km s}^{-1}$. This might be observed on a reasonable timescale, especially if the source velocity is relativistic. $\square$

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T_c increased to 90 K in $\text{YBa}_2\text{Cu}_3\text{O}_8$ by Ca doping

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THE two superconducting phases $\text{YBa}_2\text{Cu}_3\text{O}_8$ (124), containing double Cu-O chains, and $\text{Y}_2\text{BaCu}_4\text{O}_{15}$ (247), containing both single and double Cu-O chains, were originally discovered as lattice defects in the 90-K superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$ (123)¹. The synthesis of bulk superconducting 124 may be effected by a high-oxygen-pressure annealing technique^{2,3} and by reaction with ambient oxygen in the presence of alkali carbonates⁴. The oxygen stoichiometry of 124 is much more stable against high temperatures than that of 123 (ref. 2), a feature that is important for practical applications. But the lower T_c (80 K) of 124 presents a major obstacle to practical uses of this phase at liquid-nitrogen temperature (77 K). Here we report that partially substituting Ca for Y in 124 increases T_c . A superconducting transition of $\sim 90 \text{ K}$ was achieved for the composition $\text{Y}_{0.9}\text{Ca}_{0.1}\text{Ba}_2\text{Cu}_4\text{O}_8$.

We synthesized samples of $\text{Y}_{1-x}\text{Ca}_x\text{Ba}_2\text{Cu}_4\text{O}_8$ ($x=0-0.1$) using a high-oxygen-pressure technique. After mixing starting materials of Y_2O_3 , $\text{Ba}(\text{NO}_3)_2$, CuO and CaCO_3 , the samples were fired at 900°C in flowing oxygen for 12 h, and then ground. The resulting powder was compacted into a rectangular shape at 100 MPa and then lightly sintered at 800°C in an oxygen atmosphere. We repeated the hot isostatic pressing (100 MPa)

in a gas environment of argon with 20% oxygen (oxygen-HIP) twice. The samples were first oxygen-HIP-treated at 950°C for 6 h. At this stage, the X-ray diffraction patterns indicated that 124 was the major phase but broad diffraction peaks suggested a slightly disordered structure. The second oxygen-HIP treatment, at $1,050^\circ\text{C}$ for 3 h, yielded high-quality polycrystalline 124 with no secondary phases. The sample densities were 50-60% of the theoretical density.

Figure 1 shows the temperature dependence of the resistivity for $\text{Y}_{1-x}\text{Ca}_x\text{Ba}_2\text{Cu}_4\text{O}_8$. These measurements were performed using a conventional four-probe technique. As the Ca substitution increased, the magnitude of resistivity decreased monotonically. This result indicates that the substitution of Ca^{2+} for Y^{3+} introduces holes into the 124 phase. All of the samples exhibited sharp superconducting transitions ($\Delta T_c = 3-5 \text{ K}$), and the transition temperature increased with increasing Ca content x . For the sample having $x=0.1$ the onset temperature of superconductivity was 90.9 K and the resistance reached zero at 87.4 K. There is a small 'foot' on the $x=0.1$ resistivity curve, which may indicate weak links at the grain boundaries.

We measured Meissner signals (field cooling) and diamagnetic shielding (zero-field cooling) for powder samples with a SQUID (Quantum Design), in a magnetic field of 50 Oe. We estimate volume fractions of superconductivity for the samples

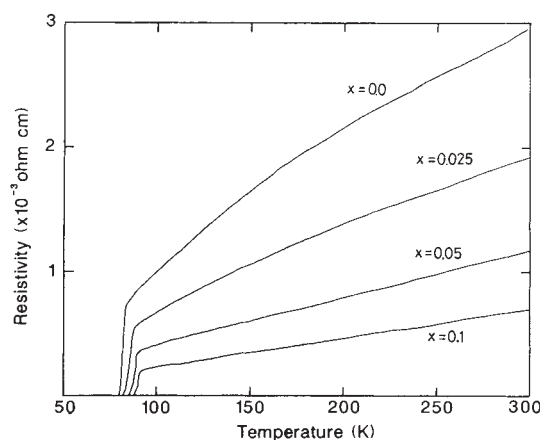


FIG. 1 Electrical resistivity of $\text{Y}_{1-x}\text{Ca}_x\text{Ba}_2\text{Cu}_4\text{O}_8$ as a function of temperature. The zero-resistance temperature for samples with $x=0.0, 0.025, 0.05$ and 0.1 are 79.6 K, 82.6 K, 84.3 K and 87.4 K, respectively.

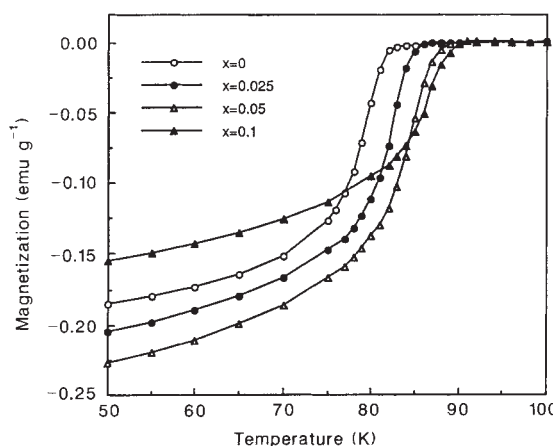


FIG. 2 Temperature dependence of magnetization for powder samples of $\text{Y}_{1-x}\text{Ca}_x\text{Ba}_2\text{Cu}_4\text{O}_8$ in a field of 50 Oe. T_c from magnetization for samples with $x=0.0, 0.025, 0.05$ and 0.1 are 83 K, 86 K, 89 K and 91 K, respectively.

to be ~30–50% from the ratio of Meissner signal to diamagnetic shielding. The Meissner signals of samples with different Ca contents are shown in Fig. 2. Values of T_c determined from the Meissner signals are in good agreement with the onset temperatures derived from the resistance drop.

X-ray diffraction patterns for samples having $x = 0.1, 0.05$ and 0.0 are shown in Fig. 3. These patterns show that the samples have the orthorhombic structure proposed in ref. 5. As the Ca content increases, the c -axis lengths slightly but the a - and b -axes remain more or less unchanged. The X-ray pattern for the sample with $x = 0.1$ includes a couple of impurity peaks with intensities <5% of the main peaks. The patterns for other samples, however, show no impurity peaks, indicating that these samples are single-phase 124. Although the superconducting transition temperature observed was >80 K, no traces of the 123 phase were present in the diffraction patterns. On the basis of the X-ray diffraction data and magnetization data (Fig. 2), the superconducting transition near 90 K must come from the Ca-substituted 124 phase.

Figure 4 shows that the thermogravimetric curves for samples with $x = 0$ and 0.1 are not significantly different. Neither sample decomposes below 800 °C. This result shows that the oxygen content of Ca-substituted 124, as well as 124 without Ca, has a high thermal stability up to a high temperature (~850 °C), and also that the 123 phase is absent in the samples. In addition, unlike 123, the materials are expected to have no orthorhombic-

tetragonal phase transformation at an elevated temperature and no twin structures.

The 123 and 124 phases are the $n = 0$ and $n = 2$ members of a homologous series of compounds having formula $Y_2Ba_4Cu_{6+n}O_{14+n}$. The increase in T_c with Ca-doping can be explained using the average charge p per $[Cu-O]^{+p}$ unit cell proposed in ref. 6. The p that maximizes the T_c of 123 seems to be 0.33 (ref. 7) whereas for undoped 124 it is only 0.25. Therefore, if one assumes that 123 and 124 have similar electronic structures, the introduction of holes by Ca-doping the 124 would be expected to cause an increase in T_c to a maximum at $p = 0.33$. In practice, T_c seems to be optimized for a doping of around $x = 0.1$ rather than $x = 0.33$, perhaps because of a non-random distribution of holes.

The 90-K 124 superconductors have a good potential for technological applications because of the relatively large margin between T_c and liquid-nitrogen temperature and because of the thermal stability of the oxygen content. To study the origin of the enhancement of T_c , synthesis of samples that are highly doped with Ca and other elements are in progress. □

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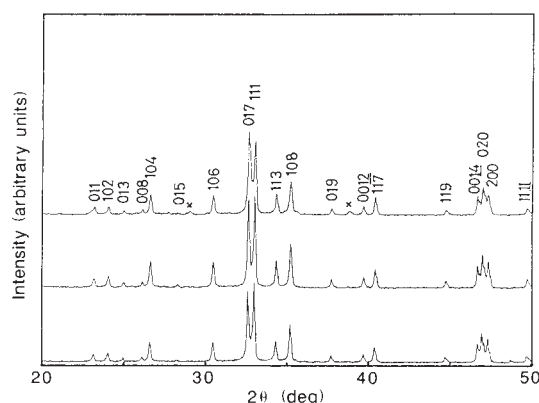


FIG. 3 Powder X-ray diffraction patterns of $Y_{0.9}Ca_{0.1}Ba_2Cu_4O_8$, $Y_{0.95}Ca_{0.05}Ba_2Cu_4O_8$ and $YBa_2Cu_4O_8$ (top, middle and bottom curves respectively). Least-squares refinement of the crystallographic unit cells yielded $a = 3.842$ Å, $b = 3.863$ Å and $c = 27.19$ Å for $x = 0.0$, $a = 3.841$ Å, $b = 3.863$ Å and $c = 27.21$ Å for $x = 0.05$, and $a = 3.841$ Å, $b = 3.864$ Å and $c = 27.21$ Å for $x = 0.1$. Impurity peaks are marked with crosses. No trace of $YBa_2Cu_3O_7$ is present.

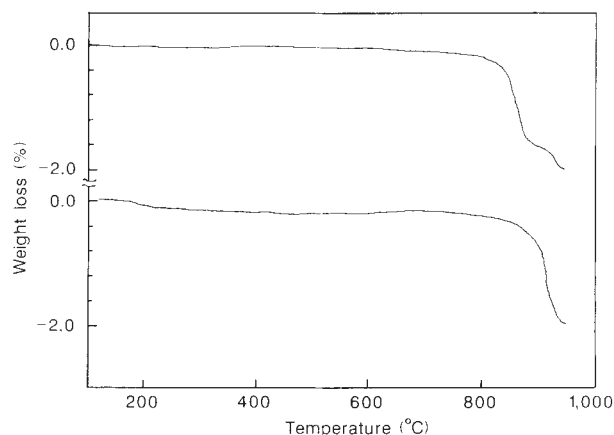


FIG. 4 Thermogravimetric curves of $Y_{1-x}Ca_xBa_2Cu_4O_8$. These measurements were carried out for samples with $x = 0.1$ (upper curve) and 0.0 (lower curve) up to 950 °C and 800 °C, respectively. The temperature was increased at a rate of 10 °C min^{-1} , in air.

The pine needle as a monitor of atmospheric pollution

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THE concentrations of toxic chemicals in the atmosphere and their deposition and long-range transport are becoming one of the more prominent issues in environmental chemistry. In 1986, a report¹ was published documenting the levels of DDT (p,p'-dichlorodiphenyl-trichloroethane) and other organochlorine contaminants in the muscle tissue of pike and herring from areas in and around Sweden. There has previously been a downward trend in residue levels but in parts of Sweden and the south Baltic during 1983–84, the ratio of p,p'-DDT to its metabolites significantly increased, indicative of freshly released DDT from a source south of Sweden. (DDT has been restricted or banned in most of Europe since the 1970s.) It was established² that an increased forest spraying program using DDT was implemented in 1984 in the southern part of East Germany. DDT can be transported over very long distances³ and in light of the above there is a need for an extensive field programme to monitor such pollution and locate its source. Here we test the hypothesis that the pine needle is a suitable monitoring matrix and delineate the extent of the DDT contamination of 1983–84. We conclude that the pine needle is a suitable monitor of atmospheric pollution and that because of the widespread distribution of the pine, it may provide time-series data for much of the Northern Hemisphere from which trends in atmospheric pollution may be discerned.

DDT and other similarly transported, persistent contaminants partition themselves between the vapour state and inert

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TABLE 1 Mean Levels of DDT, PCP, PCBs and HCH* in pine needle wax in 1984–86

Region†	tDDT			PCP			PCB			tHCH		
	1984	1985	1986	1984	1985	1986	1984	1985	1986	1984	1985	1986
A. Northern Sweden (1)	0.30	0.31	0.25	1.36	1.39	0.85	7.9	5.9	4.4	5.9	4.1	2.4
B. Norwegian foothills (2)	0.19	0.18	0.12	0.59	0.75	0.77	4.6	6.3	5.6	2.2	2.7	1.4
C. Central Sweden (11)	0.25	0.25	0.19	1.23	1.08	1.00	4.5	6.2	5.6	2.8	2.6	2.1
D. East coast of Sweden (8)	0.43	0.26	0.25	1.07	1.08	0.85	5.8	6.7	5.8	2.8	2.9	2.1
E. Northern Germany & Denmark (7)	0.65	0.63	0.30	0.48	0.53	0.42	6.8	8.1	6.8	2.0	1.8	2.7
F. Switzerland & Southern Germany (4)	0.55	0.50	0.31	0.46	0.43	0.39	6.2	7.3	4.8	2.7	2.6	1.4
G. Southern France (7)	0.47	0.29	0.25	0.23	0.25	0.51	8.1	7.9	5.6	2.6	3.3	2.9
H. Southwestern Poland (2)	‡	2.48	1.74	—	0.45	0.54	—	10.1	6.5	—	2.5	3.0
I. Southeastern Poland (2)	—	0.59	0.30	—	0.09	0.16	—	1.1	2.0	—	0.3	0.7

All levels are in ng per g of fresh needle.

* tDDT, p,p'-DDT and p,p'-DDE; PCB, total polychlorinated biphenyls; PCP, pentachlorophenol; tHCH, lindane and α -hexachlorocyclohexane.

† Numbers in parentheses are the number of sites sampled within the area.

‡ Samples from Poland were obtained for 1987; needles were not available for 1984. At one of the two West Poland sites the samples collected were from *P. mugo*.

lipophilic materials^{4,5}. Pine needles provide such a matrix as they are covered with a wax coating which, on the whole, is similar for different locales and years⁶ and has been shown to contain a number of atmospherically transported pollutants^{7–9}. Furthermore, it is easy to identify the needles from three or more years and so collect data for several years in a single sampling. Contact with rainwater and absorption of the dissolved material by the needles is likely to be slight¹⁰; dryfall particulate matter, although observed on needle surfaces, is believed to provide only a minor part of the contaminant burden of the needle wax. Translocation of the investigated compounds from the soil through the roots has been found to be a minor source of leaf residues in other plants^{10,11} but the significance of this route is not established for the pine. Diffusion within the wax layer¹² is expected to be very slow because of the highly impermeable nature of the wax subsequent to emergence. Although background concentrations of the study compounds arising from global pollution are expected to be found in the needle wax, it should also show elevated levels when releases occur locally over a sufficient period to allow absorption. We consider these conclusions, although unproven in some instances, to be reasonable and conclude that contaminant concentrations in the needle wax will largely reflect time-integrated air concentrations. Indeed, meteorological effects aside, knowledge of these concentrations and the partition coefficients for wax/air, could provide a semi-quantitative estimate of the mean air concentrations.

To test the hypothesis that the pine needle is a suitable monitoring matrix and to determine the extent of the DDT event(s) of 1983–84, we analysed samples that had been collected during the period July–September 1986. These samples were collected along a transect running from the south-west to the north-east of western Europe (see Fig. 1), the direction of the prevailing winds in most of the study area. The sample trees were selected to reflect rural and remote rather than urban or industrial conditions—the trees were 20 km or more from major urban centres and situated with open areas to their south-west; they were remote from any major highways and were sampled at 2–5 m above the ground on the south-west face of the trees. The wax coating of the needles was extracted twice with dichloromethane, treated with diazomethane, cleaned on a sulphuric-acid-containing silical-gel column and analysed using a capillary gas chromatograph with an electron-capture detector. Each sample year was analysed separately for DDT and DDE (p,p'-dichlorodiphenyl-dichloroethene), PCP (pentachlorophenol) (methylated), total PCBs (polychlorinated biphenyls) and α -HCH (hexachlorocyclohexane) and lindane (the γ -HCH isomer) for each of the 44 sample sites. Mean levels for several geographic areas grouped roughly by distance from the suspect source area are presented in Table 1. The average relative standard deviations (r.s.d.) for the group means were: 40% for tDDT, 62% for PCP, 26% for PCB and 68% for tHCH. The

years designated are those of needle growth, which is generally complete by July of the year of emergence of the needle; the needles designated 1986, 1985 and 1984 had been exposed to the atmosphere for 3–6 months, 1.5 yr and 2.5 yr, respectively, before they were collected.

The total DDT residue (tDDT) data in particular demonstrate the utility of pine-needle wax as a monitor for events such as those that are believed to have occurred in 1983–84 in the forests of East Germany. Elevated levels of 3–4 times the 'background' (the lowest means of 0.2–0.3 ng g⁻¹) were observed at Herzberg and Hamburg, sample sites closest to the presumed source, and the 1984 area of impact stretched from southern France to the coastal region of Sweden north of Stockholm—a distance of over 1,500 km. In 1985, high levels persisted in Switzerland,

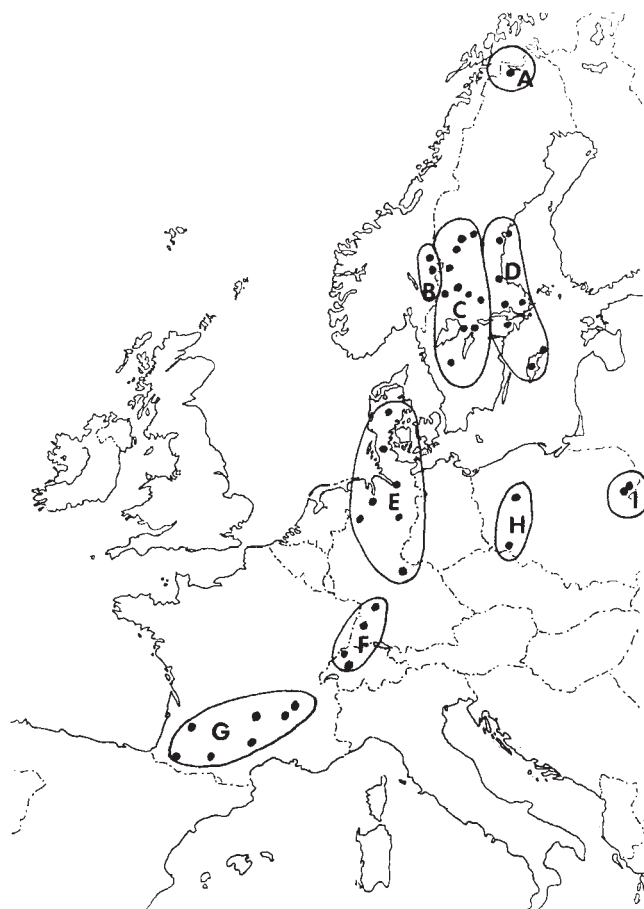


FIG. 1 Pine-needle sampling stations for the 1986 collections.

Germany and Denmark although the zone of impact was about half that of the previous year. Samples from western Poland became available in 1987 and needles from 1985 showed even higher levels there (8–10 times the 'background') than elsewhere that year. By 1986, concentrations in nearly all regions were at the background level but samples from western Poland were still 6–8 times this level. These results are illustrated in a bar graph (Fig. 2).

We did not find the expected cumulative increased in tDDT residues in needle wax from 1986 to 1984. This could have been the result of photodegradation of the pesticide (to a substance other than DDE)^{13,14} in the needle wax, to translocation into the interior of the needle or the rest of the tree¹⁵, to re-volatilization, or to lack of sorption at times other than during needle growth. Degradation seems not to be indicated because the percentage of DDT was constant (mean 68%, r.s.d. 13%) throughout the study area and period and such a high DDT/DDE ratio is indicative of freshly applied DDT. Re-volatilization of the study compounds is not indicated¹⁶ either.

In the case of the PCP results, the Swedish samples were found to contain at least twice the levels found elsewhere in Europe. This observation includes a site in the far north of Sweden where we expected levels of pollution to be lower. This overall observation for PCP is surprising because the compound has been totally banned in Sweden since 1978 (partially since 1972) although it is still in use in much of the rest of Europe including Finland. Use in the latter country could potentially contribute to the Swedish burden but the prevailing winds do not favour this. As there are no apparent changes in concentration levels over the years, we conclude that the source is still present.

The PCBs are well known pollutants in the atmosphere and elsewhere and were found in all the samples at roughly the same concentration levels (mean 6.2 ng g⁻¹, r.s.d. 26%). There were no apparent geographic or temporal trends. The HCH isomers are also well-known ubiquitous atmospheric pollutants and, as in the case of PCBs, we observe no patterns in their needle-wax concentrations. Examination of the proportion of the γ -HCH isomer (the pesticide lindane) shows that it is present in larger amounts in the French samples (mean 62%, r.s.d. 14%, 11 points) during 1985 and 1986 than elsewhere (mean 29%, r.s.d. 11%, 34 points), indicating a near-by source. As observed with tDDT residues, the ratio becomes 'normal' with increased distance and time from the apparent source.

It seems that the pine needle can provide a suitable sampling matrix for evaluating atmospheric contaminants. This seems particularly true when determining regional exposures from a single-year class and may also have relevance for trend analysis between years. The widespread distribution of the pine suggests that it may provide trend data for much of the Northern Hemisphere. In Eurasia, *Pinus sylvestris* (Scots pine), is found in most

areas north of the Mediterranean and south of the Arctic circle¹⁷. In North America, the Scots pine is an imported species but is sufficiently common that it may have utility there as an environmental biomonitor. The species *P. banksiana* (Jack pine) and *P. contorta* (Lodgepole pine), however, may be more useful for evaluating ambient conditions east and west of the Rocky Mountains, respectively. The long life of the pine, compared with other biomonitors, potentially permits sampling from the same individual over periods as long as one hundred years. □

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Atmospheric circulation during Holocene lake stands in the Mojave Desert: evidence of regional climate change

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IT is commonly thought that the climate conditions that supported lakes over a period of years in the Mojave Desert in southern California, only existed before 8,000 yr BP and that the environment has been arid since^{1,2}. Here we look at a drill core in the Silver Lake playa at the terminus of the Mojave River and find Holocene lake deposits which indicate that shallow lakes existed for at least a few decades. These deposits were radiocarbon dated at 3620 ± 70 and 390 ± 90 yr BP, corresponding to the early Neoglacial and the 'little ice age' respectively³. To identify the conditions necessary to produce these Holocene lake events we have examined the modern climate and hydrological patterns that produce ephemeral lakes in this usually arid watershed. Available data indicate that there is a link between anomalous winter atmospheric conditions over the North Pacific and Mojave River floods that produced ephemeral lakes in the Silver Lake playa and that the Mojave River filters out small to medium floods and allows only the extreme floods to reach the terminal playa and leave a record of the anomalous conditions. We suggest that the late Holocene lakes may have resulted from persistent similar atmospheric circulation patterns and winter floods.

The Silver Lake playa is the remnant of the late Pleistocene Lake Mojave^{3–6} and the present terminal basin of the closed arid watershed of the Mojave River. At 9,500 km² (Fig. 1) this watershed is the largest hydrological system in the Mojave

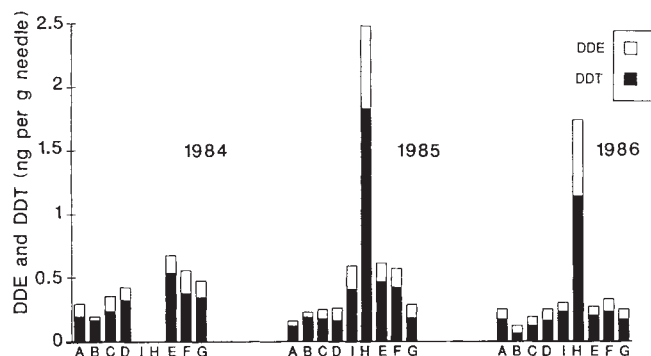


FIG. 2 Distribution of DDT and DDE in pine needles (fresh weight) (see Fig. 1 for area designations; samples were not available for areas H and I in 1984).

Desert. Present climate within the Mojave River watershed varies spatially. Its headwaters at 1,200–2,700 m in the eastern slopes of the San Bernardino Mountains (Fig. 1), are characterized by Mediterranean semi-arid to humid conditions (mean annual precipitation 500–1,250 mm), with most of the precipitation occurring between November and March. Most of the Mojave River watershed and the terminal playa region where the lakes form is hyperarid, with $<100 \text{ mm year}^{-1}$ precipitation, summer temperature often $>40^\circ\text{C}$ and consequently $>2000 \text{ mm year}^{-1}$ potential evaporation^{7,8}.

The Mojave River flow is a result of intensive winter storms at the high elevations of the San Bernardino Mountains^{8,9}. Annual discharge records (Fig. 2)¹⁰ along the Mojave River demonstrate that significant flow reaches the Afton gauging station only during those years with large discharge at The Forks in the Mojave River headwaters (Fig. 1). This is due to discharge loss to a shallow aquifer between Victorville and Afton. Hydrographs of the 1969 and 1978 winters events (Fig. 2) are characteristic of an extreme winter flood which, in turn, is related to the annual peak discharge (Y.E., Thesis in preparation). During such winter events lakes formed in Cronese Lake and the Silver Lake playas. It seems that only flood events with high peak discharge are able to exceed the loss by infiltration into the alluvial bed between Victorville and Afton (Figs 1, 2). This relation and the annual peak-discharge data¹⁰ indicate that discharge losses along the Mojave River allow only the larger floods to reach Afton. During this century a peak discharge $>300 \text{ m}^3 \text{ s}^{-1}$ at the Fork has been required for flows to reach Afton and $\sim 500 \text{ m}^3 \text{ s}^{-1}$ to reach the Silver Lake playa (Fig. 1).

We suggest that similar filtering of smaller floods occurred in the past, at least in the late Holocene. The modern conditions suggest that increased frequency of flows exceeding this natural threshold are needed and may have led to a net accumulation of water in the playas during the late Holocene.

Since 1894, eight ephemeral lakes have been documented in the terminal basins of the Mojave River (Y.E., unpublished data). Each ephemeral lake is the result of heavy precipitation during a winter storm and flooding in the Mojave River headwaters. Rainfall in several of these storms exceeded the average monthly precipitation by two standard deviations¹¹. Extreme rainfall intensity of over 25 mm h^{-1} , and as much as 250–400 mm day^{-1} , has been observed¹² in the Mojave River head-

waters at elevations $>1750 \text{ m}$. These high precipitation intensities were probably enhanced by the orographic effects of strong southerly to southwesterly winds forcing a moist Pacific air mass to cross the north-west to south-east oriented San Bernardino Mountains¹³.

The eight lake stands that were produced from these storms occupied the normally dry playa for 2–18 months (Y.E., thesis in preparation). In some of these events antecedent conditions of soil moisture in the headwaters and a wet channel along the Mojave River may have played a role as relatively large floods in the headwaters predated some of the lake-building flood events³.

To overcome the filtering mechanism of the river and to form a lake in its watershed requires a vigorous storm, unusual for southern California. We constructed the mean meteorological pattern associated with these storms and lakes. Composite monthly sea-level pressure (SLP) values (which we use because of their availability over a Northern Hemisphere grid since 1899¹⁴) and the SLP anomalies over the North Pacific and western North America were calculated for the months that included the eight cases of lake events (Fig. 3). The SLP values for each month in which a lake event occurred were subtracted from the long-term mean SLP (1947–1972)¹⁵ of the specific month to determine the monthly SLP anomaly. Although the actual flooding occurred on specific days with more intense SLP features than the monthly mean, several of the flooding events were associated with a persistent succession of storms that conformed to a particular pattern. The monthly SLP patterns provide an overall indication of the large-scale pattern that produced the particular storm or storm sequence.

During months in which lakes formed, the sub-tropical high in the eastern North Pacific weakened, giving way to an anomalously low-pressure system along the west coast of the United States. The anomalous low represents an eastward shift of the central North Pacific winter low, with vigorous storms penetrating the west coast of the United States much further south than normal. These storms produced the heavy precipitation in the San Bernardino Mountains.

The composite SLP pattern (Fig. 3) shows a tendency for a 'split' Aleutian low with higher than normal SLP in the Aleutians and an anomalously low SLP over Kamchatka. The blocking high pressure in the eastern Aleutian region during this type of

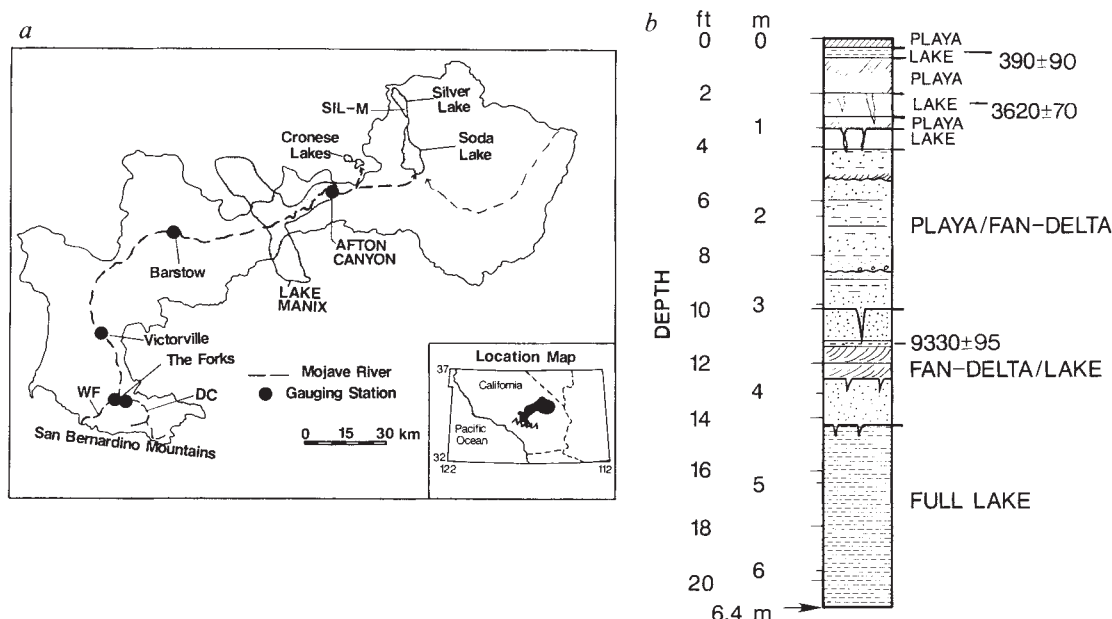


FIG. 1 a, The Mojave River drainage basin, the location of gauging stations and of core SIL-M. b, Composite lithology of core SIL-M. The date of 9330 ± 95

is from the last unit related to the late Pleistocene/early Holocene lake clays³. DC and WF are Deep Creek and West Fork, respectively.

FIG. 2 *a*, Annual discharge in downstream direction (from top to bottom) for: 1) The Forks (1905–1985)—the combined discharge of Deep Creek and West Fork, which are the two tributaries that form the Mojave River, 2) Lower Narrows near Victorville (1903–1904 and 1929–1985), 3) Barstow (1930–1985), and 4) Afton (1930–1931 and 1952–1985). *b*, Average daily discharge (left-hand scale) during December–April 1968–1969 (upper) and 1977–1978 (lower) for Victorville (solid line) and Afton (dashed line) in response to precipitation (right-hand scale) in the San Bernardino Mountains (bars), modified from ref. 9. The shaded area shows where discharge loss occurs between Victorville and Afton. Logarithmic scales are used for both discharge and precipitation.

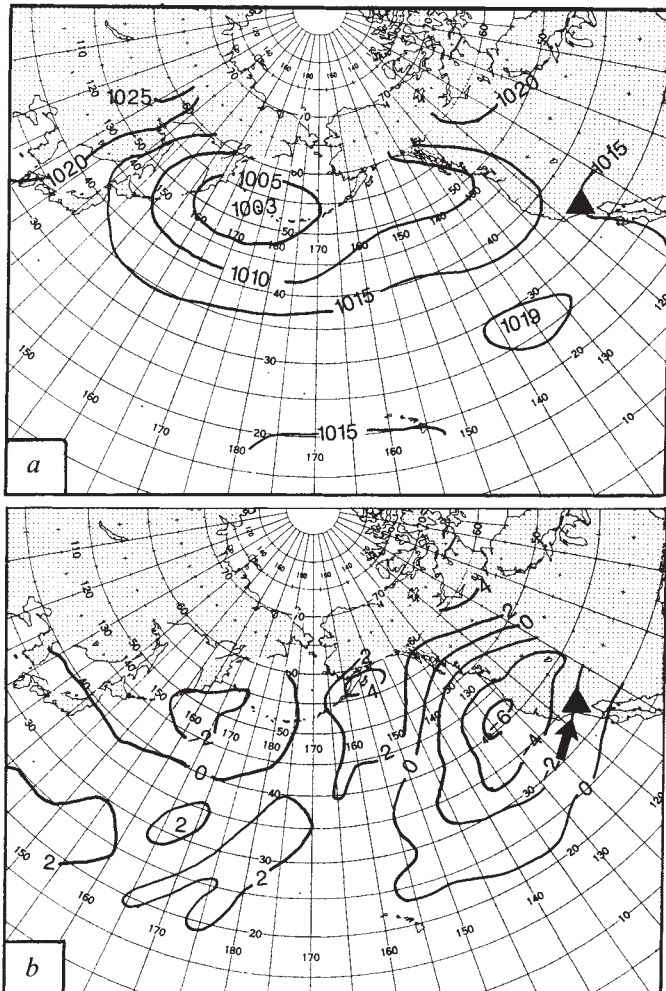
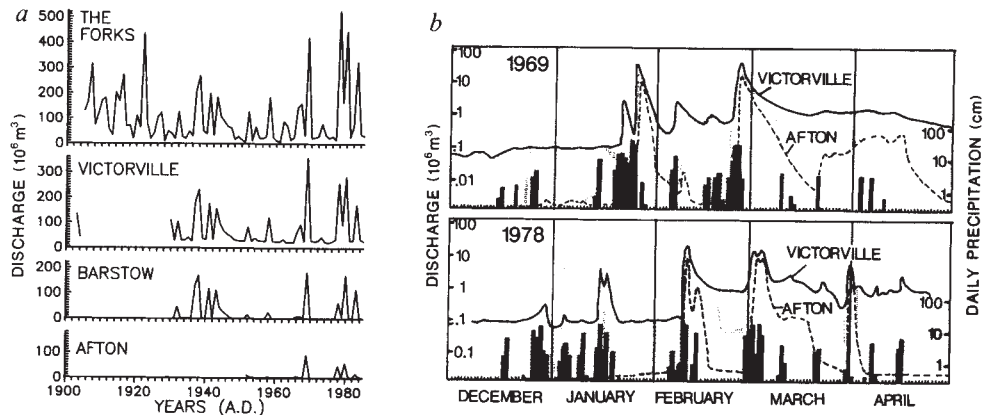


FIG. 3 *a*, Composite North Pacific sea-level pressure for the eight months with lake-building flood events. Triangle indicates location of the San Bernardino Mountains. *b*, Composite SLP anomaly for the same eight months. The arrow shows the anomalous component to the mean wind direction that results from the anomalous SLP. The enhanced southwesterly wind advects moist warm Pacific air over the San Bernardino Mountains and the negative pressure anomalies indicate the necessary vorticity for extreme precipitation events.

event is also borne out by inspection of daily maps. This synoptic pattern has been diagnosed as a potent flood-producing condition in California¹³. The Mojave River flood and lake events are symptoms of this pattern.

The strong negative anomaly of -6 mbar at 40°N , 130°W (Fig. 3) represents 1.5–3.0 standard deviations from the long-term monthly mean (depending on the specific winter month in which the flood occurred). Other studies of winter and monthly average precipitation and stream flow have shown this condition to favour heavy precipitation and stream flow in California^{16–18}.

Individual 500-mbar and 700-mbar height maps, satellite images, and previous studies^{17,19} for the winter and the months of the floods in 1969, 1978, 1980 and 1983 floods, reveal two recurrent synoptic patterns. The most frequent pattern featured an unusually deep trough or cut-off low pressure offshore from California and a blocking high pressure in the Gulf of Alaska with a southerly displaced storm track entering California. In some of these cases, such as the 1969 storm²⁰, moisture and strong winds of these storms were assisted by the activity of the sub-tropical jet stream. A second pattern that emerged, was a sequence of storms embedded in strong southerly-displaced westerlies that spanned the entire eastern North Pacific. This pattern occurred during the 1980 and 1983 events.

There is evidence²¹ that ocean surface boundary conditions may have played a role in the development of these storms. Highly persistent patterns of sea-surface-temperature (SST) anomalies were found in the North Pacific during the winters of 1969, 1978 and 1980, in which lakes were formed. Although probably not a necessary condition, such winter-SST-anomaly patterns may increase the frequency of extreme storm episodes.

Water-budget calculations suggest that an order of magnitude increase in the modern average annual discharge at Afton Canyon (from $9.4 \times 10^6 \text{ m}^3$ to $90 \times 10^6 \text{ m}^3$) is required to maintain a lake in the terminal playas. Water volumes similar to the latter discharge have been measured during the short-term extreme historical floods. Decreasing evaporation alone will not maintain a lake without the large runoff increase³. Because the extreme floods are relatively rare (recurrence intervals of 34 years in Afton), the modern average annual river discharge is not representative of the actual volumes that can reach the playas during the large floods. If the volumes of the modern extreme floods are used as the total annual input over a series of years in the lake's hydrological budget calculations, a lake can be established in Silver Lake³. An increase in the frequency of flood events with magnitudes similar to the modern largest floods would therefore result in a perennial lake in the Silver Lake playa. This may have caused the formation of the late Holocene lakes.

Analyses of tree-ring data from southern and central California^{22,23} indicate that more-frequent wet conditions occurred in

the late 1500s and early 1600s at the same time that the 'little ice age' lake was maintained in Silver Lake basin²³. The high-frequency, high-magnitude annual precipitation and stream flows that have been inferred for that period in southern California^{22,23}, support our suggestion of increased frequency of the high-magnitude precipitation and runoff events during the 'little ice age' lake stand. As the lake-building storms of the past century were all vigorous eastern North Pacific cyclones that occurred during the winter, we postulate that similar North Pacific disturbances were more frequent during the late 1500s and early 1600s. Reconstructions of North Pacific SLP from tree-ring chronologies²⁴ showed that the early 1600s to have had a high frequency of the pattern of North Pacific SLPs similar to the SLP pattern of the wet lake-forming winter of 1978. LaMarche²⁵ suggested that persistent 'little ice age' and earlier climate anomalies can be explained by changes in the frequency of anomalous circulation types. One of these types was characterized by upper-level trough centred over the Pacific Coast of North America.

We suggest that extreme modern hydrological events in the Mojave River watershed serve as an indicator of particular atmospheric forcing patterns over the North Pacific and thus can be used as analogues for conditions during the Holocene when lakes formed in the watershed. An increased frequency of these extreme events can explain the observed lake deposits in the Silver Lake playa. □

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Storm wave reactivation of a submarine landslide

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SUBMARINE landslides move large amounts of sediment and are often hazardous to offshore installations. Marine surveying techniques effectively define landslide locations and geometries (for example, refs 1–4) in various underwater geological settings, including deltas, fjords and earthquake-prone areas^{1–5}. Movement rates and mechanics are generally inferred from indirect evidence, such as cable breaks and damage to sea-floor structures (for example, refs 6–8) but actual measurements of landslide behaviour are sparse. Using bottom-deployed pressure sensors, tiltmeters and accelerometers, we have collected data on sea-floor landslide reactivation involving sediment collapse and remoulding on the submarine slopes of the Huanghe delta, China. The sensors were emplaced into sea-floor clayey silts on 4 October 1987 and recovered on 16 October after the passage of three severe storms.

The Huanghe delta provides an ideal natural laboratory for the measurement of depositional and post-depositional deformations^{3,9} of the sea floor. The delta has very high deposition

rate¹⁰ and the delta front is subjected to storm waves. Further, the shallow water facilitates the deployment of instruments.

Post-depositional slope failure creates collapse depressions and silt flows on the underwater Huanghe delta in water depths of 4–15 m and over gradients of 0.3–0.4° (Figs 2, 3). Roughly circular collapse depressions (0.5–1.0 m depth) occur mainly on

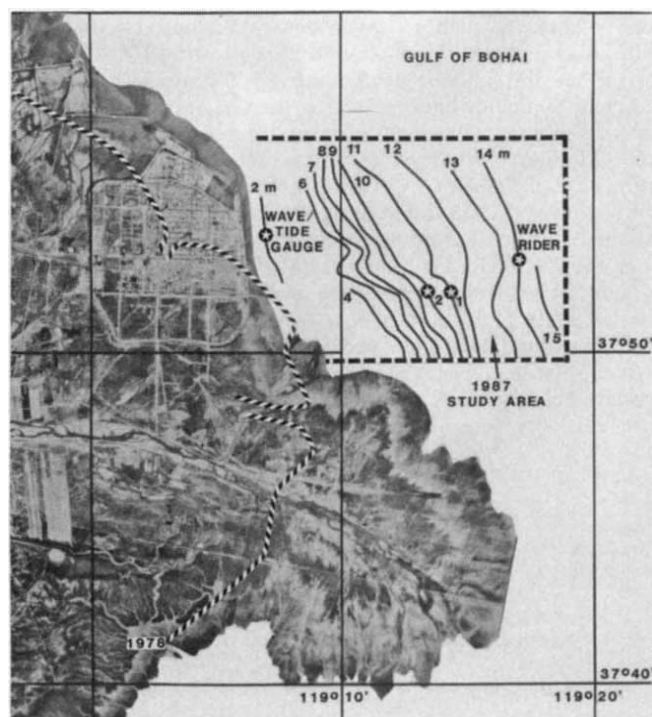


FIG. 1 Location of *in situ* measurement on the north-east flank of the Huanghe delta. Shoreline position is the 1987 position; former (1978) shoreline is outlined.

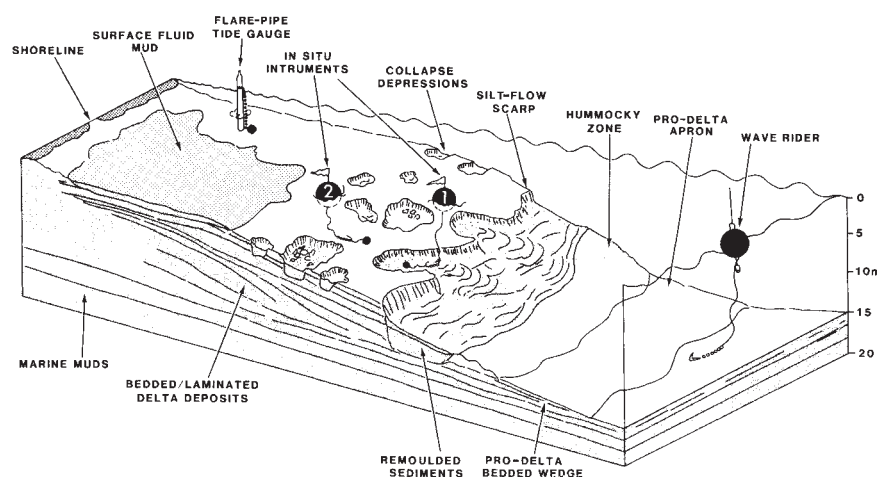


FIG. 2 Schematic representation of instability features on the northeast-facing slopes of the Huanghe delta and the deployment of instruments.

the upper delta in water 4–8 m deep. Between 9- and 11-m water depth, numerous finger gullies 100–500 m wide lead downslope towards a pronounced silt-flow scarp. This scarp is 1–2 m high and bounds the upslope edge of a highly deformed area, extending around the delta front in water depths of 11–14 m. Farther downslope blocky, 'ropey' areas of remoulded sediments are widespread. The collapse depressions and silt-flows are acoustically transparent, remoulded sediment areas on 3.5-kHz profiles, as compared to parallel bedding in neighbouring undisturbed areas.

The bottom features and sediment geometries were previously ascribed to liquefaction of rapidly deposited silts under the influence of storm-wave-induced bottom stresses³. To test these concepts and obtain *in situ* data on deformational processes, we deployed instruments along an offshore-onshore transect at the beginning of the autumn storm season (Figs 1, 2). Our approach was to measure waves entering from the maximum fetch while simultaneously monitoring bottom response inside and outside areas showing deformation. An inshore wave-tide pressure gauge was attached to an oil-field flare pipe in a water depth of 2 m. An offshore wave-rider was anchored in the lower delta in 14-m water depth (Figs 1, 2). Following 100-kHz side-scan sonar and 3.5-kHz profiler surveys for site selection, two sediment-dynamics packages were inserted into the uppermost 1 m of sediment. One package was located within a silt-flow gully (1 in Fig. 2), where the thickness of the remoulded sediment to the base of the silt flow was 3.8 m in a water depth of 11.0 m. The sediment texture from coring within the silt flow was 62.3% silt and 37.4% clay. Another package was placed 1-km upslope from the first package in an area of undisturbed sea floor underlain by 6–7 m of parallel-bedded delta-front sediments in a water depth of 9.5 m (2 in Fig. 2). The sediment texture from a core averaged 49.4% silt and 50.5% clay.

Each instrument package contained a compass, a three-axis accelerometer, sensors for *x* and *y* tilt, and water column and pore-water pressure sensors. The water-column pressure-sensor

port was located at the top of the package, whereas the pore-water porous tip was at a depth of ~1 m below the mudline. The packages were designed to record accelerations and water-column pressures at a 1-s sampling interval over a 15-min period every 3 h. Eight sequential readings of tilt, compass and pore-water pressure at 1-s intervals were also made every 3 h. The specific gravity of the packages was 3.7, or about twice the density of the sea-floor sediments.

Three severe autumn storms occurred between 5 and 15 October, with winds from the north and northeast at sustained speeds ranging from 15 to 25 m sec⁻¹. Our research vessel, the *Huanghe 86*, sheltered inshore, close to the flare pipe, on 6 and 7 October, and also continuously from the evening of the 11 October until early on 16 October. A larger vessel, *RV Dong Fang Hong*, anchored offshore from the delta in water depths of 11–15 m, making visual observations of wave conditions in support of wave-rider records.

During the storms significant wave heights at the wave rider reached 2.5 m, with a period of 8.2 s. The first storm occurred on 6–7 October and was followed four days later by two more closely spaced storms (Fig. 4a).

Wave energy was dramatically attenuated across the delta front in all three storms. For example, the significant heights at the wave rider and inshore flare pipe for 12 and 14 October were 2.1 and 0.6 m, and 2.5 and 0.8 m, respectively. Wave periods diminished from 8.2 s offshore to 7.1 s inshore. In general, there was an observed 80% reduction in wave energy across the delta front.

The instrument package in the featureless, undisturbed area (2 in Fig. 2) remained virtually stationary during all three storms, except for some very low amplitude (~1 cm) oscillatory motion (periods 6–8 s) of sediments in response to cyclic loading by the waves. Comparison of side-scan sonar records made before and after the storms confirmed that the featureless area at location 2 remained unchanged.

In contrast, the sensors in package 1 revealed three distinct

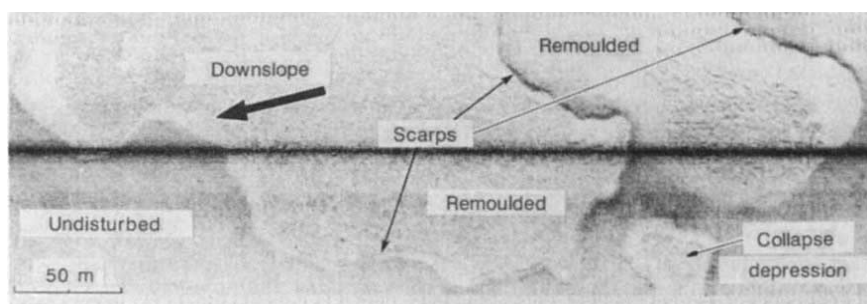


FIG. 3 Side-scan sonar swath illustrating typical silt flow and collapse-instability features on the Huanghe delta front.

periods of bottom sediment motion (Fig. 4c-e) because of reactivation of the silt flow during the storms. The instrument package underwent several types of motion. For example, the difference between bottom pressure signals from packages 1 and 2 (Fig. 4c) indicates the sinking of package 1 into the bottom. On 5 October, upon deployment, package 1 experienced initial settling. The package sank farther over a period of ~6 h on 6 October and larger displacements followed on 11-12 and 14-15 October. Accompanying these sinking movements were three discrete tilting events, beginning on 6 October for ~15 h, on 11-12 October lasting 27 h and on 14-15 October for 21 h. During these storms the package sank >1 m into the mud and tilted a total of 150° (Fig. 4d). The beginning of each package motion event coincided with increasing wave energy (Fig. 4a, c, d). The initiation of movement coincided with wave-bottom pressures at packages 1 of 4.5 kPa on 6 October, 6.5 kPa on 11 October and 9.3 kPa on 14 October. Motion ceased toward the end of each storm when wave-bottom pressures dropped to less than 2-3 kPa (Fig. 4a).

The details of pore-water pressure changes, recorded by the sinking and tilting package, are complex. Tidal variations of pore pressure were detected, along with mean changes. To extract the mean changes (Fig. 4b) from the total record, the mean bottom pressure signal was subtracted from the pore-pressure signal in package 1. During the first storm, the relative pore-water pressure increased late on 6 October and then decreased early on 7 October. Another relative pore-water pressure rise began on 10 October, before the arrival of the next storm. The pressure then declined throughout that storm and remained negative, declining irregularly until the package was retrieved. Because of the movement of the package, any reliable correlation between pore-water pressure, movement and storm activity was lost. After the first movement event, when pore-water pressure increased initially and then dropped as movement began, the pore-pressure data are erratic and are not considered accurate.

Vertical oscillations of the near-surface sediments and the instrument package also occurred during the first two storms (Fig. 4e). By the third storm, the highly tilted attitude precluded further reliable measurements of the oscillations. During the first storm, bottom motion was detected 3 h before the tilting and sinking were initiated and it occurred simultaneously with the increase in pore pressure (hour 18 on 6 October). The bottom motions reached maximum amplitude at 0300 and 0600 on 7 October, 3 and 6 h after the peak in the bottom pressure was reached.

Although the packages showed oscillatory and vertical motion, actual cumulative horizontal transport was not determined owing to limitations in navigation accuracy. Comparison of sonar coverage of surface silt flows before 6 October and after 15 October showed local roughening, but the overall outlines of the silt flows were unchanged after the three storms.

The data clearly show that existing landslide features experienced renewed and repeated movement without enlargement or upslope retrogression. These observations contradict the concept that failed areas should be more stable because of reduced slope, dewatering and consolidation. Similar evidence for activity of existing collapse features was acquired from the Mississippi delta front, in which wave and pore-pressure sensors foundered within the sediment¹¹.

The Huanghe delta data also show that existing landslide features are more susceptible to renewed movement than surrounding undisturbed areas. First-time slope failure apparently requires greater applied stresses than are necessary to reactivate already failed areas, at least within the stress ranges measured during the experiment. Perhaps the very small differences in sediment properties at site 1 and site 2 (for example, 10-15% contrasts in clay and silt contents) are sufficient to account for the different behaviour.

The data show that landslide reactivation movement was not

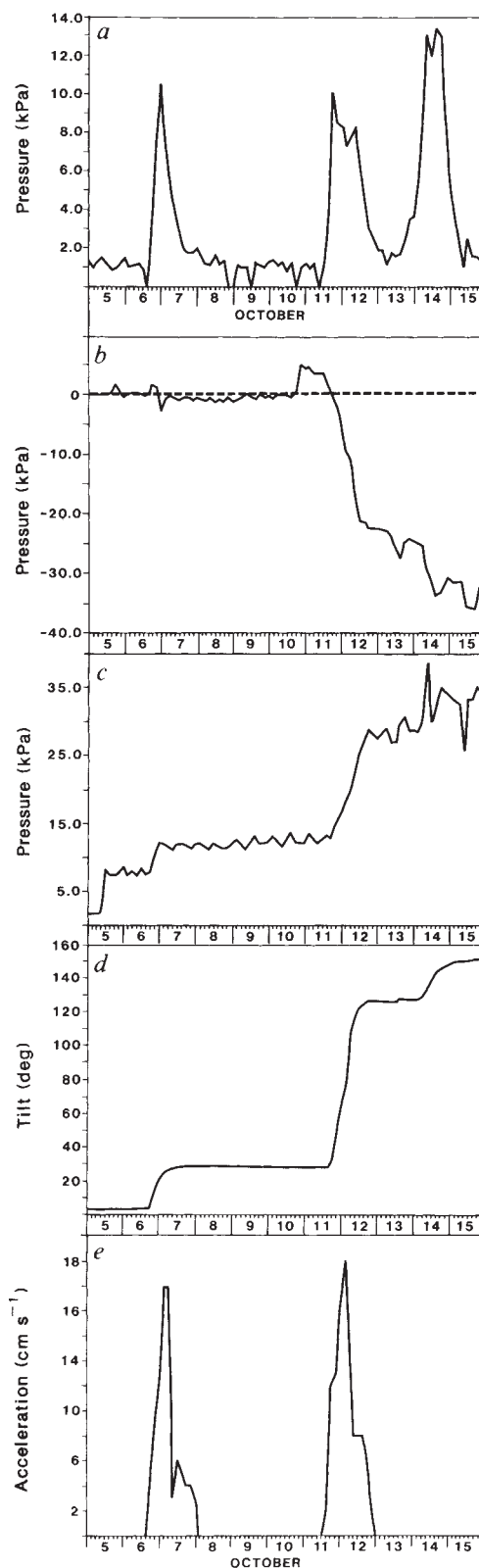


FIG. 4 *In situ* data showing a, wave-bottom pressure from site 2 and time-series measurements of b, pore-water pressure c, residual bottom pressure d, tilt (y-axis) and e, wave-induced bottom accelerations (z-axis) within silt-flow feature at site 1. Wave data (a) is maximum amplitude during a 3-h period.

abrupt and catastrophic, but gentle and gradual occurring over many hours. This behaviour is in strict contrast to that inferred from cable-break data in other documented cases of first-time failures (for example, refs 6, 7).

The measured attenuation of wave energy exceeds that which would be predicted from conventional wave theory. Because there is little motion of the bed in undisturbed areas of the delta front, we conclude that the energy sink is in the oscillatory motion of sediments in the collapsed areas. A similar pattern of wave attenuation across the Mississippi-delta mudslide region has been recognized¹².

Each reactivation event involved loss of bearing capacity of the sediment within the feature, permitting the negatively buoyant package to sink. At the end of each wave-loading period, sinking ceased, suggesting some regain in bearing strength. This behaviour might be interpreted as a type of liquefaction in which the sediment progressively becomes more dense, but then the amount of reactivation would be expected to decline. Laboratory analyses of time-dependent strength changes on samples taken from inside the feature at site 1 show thixotropic characteristics.

Submarine magma degassing and explosive magmatism at Macdonald (Tamarii) seamount

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MACDONALD seamount is an active volcanic centre located at 29°98' S, 140°25' W in the south-central Pacific. Since its discovery¹ in 1968, a number of expeditions have surveyed and dredged rocks from the seamount. During a Scripps Institution of Oceanography expedition in October 1987, the first direct observation of volcanic activity at Macdonald was made when a small eruption occurred spewing forth scoriaceous basaltic rocks which briefly floated to the sea surface. The rocks were accompanied by huge bubbles of volcanic gas that created large sea surface slicks (H. Craig, personal communication). Similar activity at Macdonald was observed by a joint French-German team in January 1989 using the submersible *Cyana*². Here we look at the radio-geochemical features of the lone rock recovered from the October 1987 eruption. We present analyses of U-series nuclides from the first observed eruption of Macdonald (also known as Tamarii) seamount with interpretations focusing on the timescales of magmatic events and the implications of uranium-series data for magma degassing. This highly gas-charged magma lost all of its volatile ²¹⁰Po upon eruption. Based on this, estimates of the fluxes of other volatile elements to sea water, which may contribute significantly to their oceanic budget, are calculated. High (²³⁰Th/²³²Th) and ⁸⁷Sr/⁸⁶Sr ratios, common signatures of altered crust, suggest that the explosive nature of the Macdonald volcanism results partly from the sealing of magma conduits by seawater circulation.

Macdonald seamount sits on Eocene age Pacific lithosphere³. Based on a regression of ages for the Austral-Cook islands, Macdonald seems to be the current active site of that linear hotspot chain^{4,5}. The volcano rises ~1,800 m from the sea floor and has a small summit plateau (100 m × 150 m) about 40 m below sea level (b.s.l.) with one 50 m × 30 m pinnacle at 27 m b.s.l.⁶. In 1983, scuba divers identified a rift on the summit plateau consisting of fresh walls with small (6-m-high) spatter cones of scoriaceous lava on either side⁶. Although no volcanic activity had been observed directly, the summit had apparently

Undrained strengths, from fall-cone tests on remoulded sediments increased by 90% within 30 h, with no change in water content. Subsequent remouldings of the sample repeated the pattern of dramatic strength loss and regain. □

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enlarged in the eight years since previous surveys⁷, adding some 20 m to the pinnacle⁶.

Macdonald seamount was originally discovered as a source of teleseismic (T) waves associated with gas bubbles bursting in the water column during eruption of the volcano¹. T-wave records registered by the Polynesian seismic network provide a detailed record of eruptions since 1968^{2,6}. From 1968 until 1977, the volcano was quiescent. A ten-year period with 1-24 days of activity per year followed. In mid-1987, the volcano entered a very active phase and, since then, has been erupting about 50% of the time. The T-wave record shows that more than half the eruptions start with explosive outbursts. This is in contrast to the less strombolian eruptive style usually inferred for submarine volcanism.

The rock sample recovered from the 11 October 1987 eruption, which floated to the surface and was still warm when collected, is a scoriaceous alkali basalt measuring about 30 × 15 × 15 cm. It contains 40-50% vesicles by volume in a glassy matrix. Trace amounts of very small (0.1 mm) unzoned plagioclase (An₈₅) and olivine (Fo₆₅) phenocrysts are present. The glass composition, determined by electron microprobe analysis, is 44.5% SiO₂, 4.48% TiO₂, 5.4% MgO and 5.38% Na₂O + K₂O. Previous expeditions have recovered mostly alkali-olivine basalts of similar composition and gabbros from the summit and flanks of the seamount, suggesting that the shield-building stage is essentially over (ref. 8 and M. Seguin *et al.*, unpublished data). Compositions of these rocks indicate that their parental magmas experienced fractionation in a crustal magma chamber, which has been identified with magnetic surveys⁷.

The activities of all ²³⁸U-series nuclides are equal in a closed system, a state known as secular equilibrium. Disequilibrium between any pair of these nuclides in an erupted lava gives direct information about the timescale and extent of chemical fractionation during the magma lifetime. This is because the nuclides are distinct chemical species that can be separated from each other by fractionation and because secular equilibrium is re-established according to the half-life of the daughter nuclide. In general, disequilibrium is distinguishable from secular equilibrium over a period of ~5 half-lives of the daughter isotope. Following convention, we denote activities by parentheses.

Of particular interest in freshly erupted volcanics are the systematics of very short-lived ²³⁸U daughter nuclides such as ²²²Rn, ²¹⁰Pb, ²¹⁰Po and ²¹⁰Bi. These elements are all volatile to varying degrees during magmatic processes, and their half-lives (3.8 days, 22 yr, 138 days and 5 days, respectively) are especially suitable for investigating magma degassing. Because each degassed volatile nuclide will follow a predictable ingrowth

pattern relative to a non-volatile parent, its initial concentration in the magma can be determined by monitoring the temporal changes in its activity in a freshly erupted rock. Thus a major difficulty in calculating the degassing efficiency of a given species, estimation of its initial concentration in the magma, is overcome by direct measurement.

The vapour supersaturation and subsequent degassing of Rn, Po and Bi nuclides from freshly erupted subaerial volcanics are well known from studies of both the rocks themselves⁹⁻¹¹ and filtrates of volcanic plume materials¹²⁻¹⁷. In most cases both ²²²Rn and ²¹⁰Po are completely degassed from freshly erupted basaltic lavas, and ²¹⁰Bi is ~20% degassed. Little or no ²¹⁰Pb degasses. In addition, volcanic ash and plume condensates have large (²¹⁰Po/²¹⁰Pb) ratios, up to 120 (ref. 17), and large enrichments of ²²²Rn are observed in the plume relative to normal air¹².

The Macdonald sample is the first freshly erupted submarine volcanic material to become available for U-series measurements. As in the case for subaerial volcanics, ²¹⁰Po was 100% degassed from the Macdonald magma on eruption (Fig. 1). In addition, ²¹⁰Po measurements on sea water collected over the erupting volcano show qualitatively that much of the polonium condensed there. The seawater sample contained ~5 Vol% suspended plume material when collected; by the time of analysis this material had dissolved in the acidified seawater (H. Craig, personal communication). The ²¹⁰Po concentration, extrapolated to the time of eruption, is 66 d.p.m. dm⁻³, which is significantly larger than open-ocean surface seawater values of 0.04–0.09 d.p.m. dm⁻³ (refs 18, 19). Furthermore, we recently analysed similar material collected by the *Cyana* expedition in 1989 during eruption of Macdonald. The dried sample contains 970 ± 65 d.p.m. g⁻¹ ²¹⁰Po, extrapolated to the time of eruption, or ~500 times by weight the amount in the analysed rock.

In the Macdonald sample (²¹⁰Pb/²²⁶Ra) = 0.85. The two simplest explanations for this observation are the loss of ²¹⁰Pb by degassing or the addition of ²²⁶Ra (possibly from recently altered crust) to the magma. The required 15% degassing loss is, however, much larger than would be expected from estimates of the Pb degassing efficiency, which are ~1% (ref. 11). This hypothesis is also inconsistent with (²¹⁰Po/²¹⁰Pb) ≈ 100 in the *Cyana* slick material (G. McMurtry, personal communication). The addition of Ra is also unlikely because it would imply an unusually low (≤1) indigenous (²²⁶Ra/²³⁰Th) for the magma before Ra addition. The only intermediate nuclide between ²²⁶Ra and ²¹⁰Pb is gaseous ²²²Rn; thus it is also possible that Rn loss caused the deficit. The mechanism could include either sustained partial ²²²Rn degassing from the whole convecting magma chamber over about the past hundred years or complete degassing from a smaller reservoir which was mixed with a fresh undegassed magma just before eruption. Such degassing would affect Rn and other gases such as Ar, but probably would not affect volatile metals, which are lost only upon eruption.

The volatile metals lost from an explosively erupting magma such as that at Macdonald would include Bi, Cd, Ir, Se, Hg and As^{20,21} as well as less volatile elements such as Pb, Cu and Zn, some of which have concentrations as low as 10⁻¹² M in sea water. Although it is difficult to extrapolate from a single volcano to an ocean-wide flux, it is nevertheless worthwhile to make the first estimates of the amount of volatile-metal addition to sea water during explosive submarine volcanism at Macdonald. Such input is completely different from the hydrothermally mediated fluxes of various elements during less explosive ridge-crest and (deep) seamount eruptions. The estimated flux of ²¹⁰Po in d.p.m. yr⁻¹ to the ocean from alkalic volcanism at Macdonald is: $(F_{Po}) = V(^{210}Po)_0 \rho E_{Po} / \Delta t$, where V is the volume of magma produced, $(^{210}Po)_0$ is the average activity of ²¹⁰Po, ρ is the approximate magma density, E_{Po} is the Po degassing efficiency and Δt is the time interval. Based on observations of ocean islands today, reasonable estimates for the volume and duration of post-shield alkalic volcanism are ~5% of the volcano volume and 300,000 yr. From this we infer a present-day

effusion rate at Macdonald of 1.67×10^{-5} % of the volcano volume per yr. Using 4.34×10^{12} m³ for the total volume of Macdonald, $(^{210}Po)_0 = 1.43$ d.p.m. g⁻¹, $\rho = 2.9$ g cm⁻³ and a degassing efficiency of 100% ($E_{Po} = 1$), it is estimated that the average present-day flux of ²¹⁰Po from Macdonald is 3.0×10^{12} d.p.m. yr⁻¹.

With some further assumptions, it is possible to estimate the present-day flux of other metals from Macdonald to sea water. For subaerial volcanoes, Lambert and coworkers²² used $E_{Pb} = 0.01$ to calculate the volcanic F_{Pb} using plume ²¹⁰Po measurements and $E_{Po} = 1$. All other element fluxes were then normalized to the Pb flux using the equation $F_X = F_{Pb}(E_X C_X)/(E_{Pb} C_{Pb})$, where C_X is the average concentration of the element of interest in the erupted rocks. Using published values of E for Pb, Cd, Zn, Cu and Bi²³, an average of 0.3 d.p.m. ²¹⁰Pb per µg Pb in Austral-Cook alkali basalts (inferred from data in ref. 24) and average concentrations for these elements in similar rocks, we use the same approach and obtain approximate fluxes from Macdonald (in 10⁻⁴ Gg yr⁻¹) of 1, 0.4, 2, 6 and 0.6 for these elements, respectively. These are consistently 0.004% of the calculated global subaerial volcanic aerosol output²² and range from 0.002% (Cu) to 3×10^{-7} (Pb) of the calculated anthropogenic atmospheric input (significant only within the last century) of these metals²⁵. Although these fluxes seem relatively small by comparison, neither the oceanic inputs nor the river fluxes from these latter sources are well known, and all fluxes have considerable associated error. Obviously, the total volatile input to sea water depends upon the unknown number of active Macdonald-like volcanoes but it seems to be a minor component of the total budget for these elements.

Regardless of the global budgets, explosive submarine volcanism must have a large impact on the local budget for these elements, especially if the volcanoes are located in areas isolated from continental inputs. For elements having short oceanic residence times, one would expect pulses of scavenged-volatile-element enrichment in sediments near seamount volcanoes

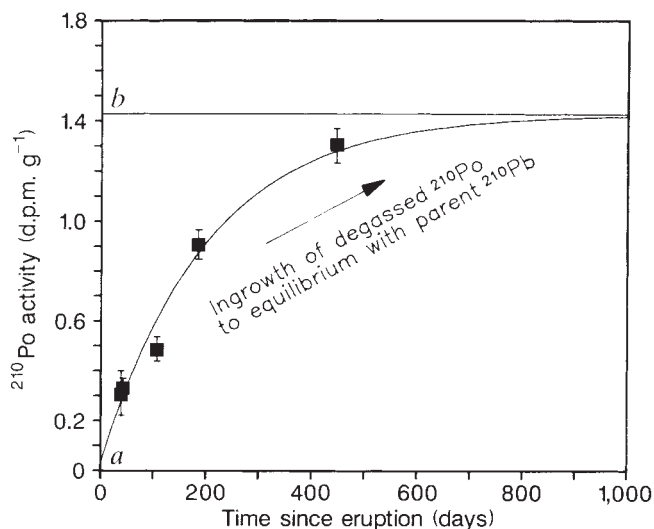


FIG. 1 The ingrowth of (²¹⁰Po) (d.p.m. g⁻¹) in the analysed rock as a function of time since the eruption. The exponential curve is a best fit to the data and shows that the initial ²¹⁰Po activity upon eruption a was 0.02 ± 0.08 d.p.m. g⁻¹ and the ²¹⁰Pb activity to which it is growing b is 1.4 ± 0.1 d.p.m. g⁻¹. The effect of the possible partial degassing of ²¹⁰Bi (the only intermediate nuclide between ²¹⁰Pb and ²¹⁰Po) is only apparent in the first 25 or so days after eruption. Therefore, the effective ingrowth of degassed ²¹⁰Po to equilibrium with grandparental ²¹⁰Pb is governed by the equation: $(^{210}Po)_t = (^{210}Po)_0(e^{-\lambda t}) + (^{210}Pb)(1 - e^{-\lambda t})$ where λ is the ²¹⁰Po decay constant and t , in this case, is the time since eruption. (²¹⁰Po) was measured by autoplating the metal on to Ag planchets from solutions of the dissolved rock³⁷. The error bars show 2σ counting uncertainty.

TABLE 1 Isotope data for Macdonald seamount

Th (p.p.m.)	4.3±0.3	Th/U	2.6±0.5
U (p.p.m.)	1.7±0.1	[Th/U] _s	2.1±0.2
^{(238)U}	1.25±0.07	^{(230)Th} / ^{(232)Th}	1.5±0.1
^{(234)U}	1.30±0.07	^{(234)U} / ^{(238)U}	1.04±0.06
^{(232)Th}	1.04±0.05	^{(230)Th} / ^{(238)U}	1.2±0.1
^{(230)Th}	1.5±0.1	^{(226)Ra} / ^{(230)Th}	1.12±0.09
^{(226)Ra}	1.70±0.07	^{(210)Po} / ^{(226)Ra}	0.84±0.08
^{(210)Pb}	1.4±0.1	^{(210)Po} / ^{(210)Pb} _i	0.01±0.04
^{(210)Po} _{sw}	66±20	¹⁴⁴ Nd/ ¹⁴³ Nd	0.51283±0.00001
^{(210)Po} _{slk}	970±65	⁸⁷ Sr/ ⁸⁶ Sr	0.70366±0.00003

Activities (in d.p.m. g⁻¹) and activity ratios of U-series nuclides in fresh Macdonald seamount basalt. U and Th isotopes were measured by high-resolution alpha spectrometry (see, for example, ref. 31). (²²⁶Ra) was measured by the ²²²Rn-emanation method (see, for example, ref. 36). (²¹⁰Po)_{sw} is the average initial activity (d.p.m. dm⁻³) in seawater collected during the 11 October 1987 eruption based on measurements made on 2 February and 23 February 1988. (²¹⁰Po)_{slk} is the initial activity in slick material collected during the 1 February 1989 eruption based on a single measurement made on 13 June 1989. Sr and Nd isotopic ratios were measured by thermal-ionization mass spectrometry. Th/U is the value observed in the rock and [Th/U]_s is the source value calculated from (²³⁰Th/²³²Th).

independent of any volcanic ash component. The occurrence of such horizons could help provide dates for alkalic magmatic activity at now dormant volcanoes, for example along a hot-spot seamount chain.

In addition to the isotopes already discussed, we measured other U-series nuclides (Table 1) which help to characterize the volcanism at Macdonald. We note that the activity ratios (²²⁶Ra/²³⁰Th) and (²³⁰Th/²³⁸U) are within the range of values for other ocean island basalts (OIB) (refs 26, 27 and our unpublished data); the ²²⁶Ra excess constrains the crustal residence time of the magma to ≤8,000 yr, and (²³⁰Th/²³²Th) is high and considered in isolation, implies a low value (~2.1) of Th/U in the source. This is more similar to values characteristic of the mid-ocean-ridge basalt (MORB) source than of the OIB source (refs 26, 28–31). On the Th–Sr isotope diagram, the Macdonald seamount datum falls far above the broad array defined by oceanic rocks³², suggesting that either the Sr or Th isotopic ratios have been affected by secondary processes. Comparison of our Macdonald seamount Sr and Nd data (Table 1) with those from other nearby eastern Austral–Cook islands^{24,33} suggests that ⁸⁷Sr/⁸⁶Sr is indeed high for the measured Nd isotopic composition and that the source Sr isotopic ratio may be closer to 0.7028. If this is so, the Sr–Th isotope correlation indicates that (²³⁰Th/²³²Th) should be close to 1.25, considerably lower than the measured value but still far above the OIB range, indicating a truly depleted source for Macdonald.

The process most likely to cause the secondary isotope shifts is the incorporation of altered oceanic crustal materials, because both U concentration (and hence ²³⁰Th) and ⁸⁷Sr/⁸⁶Sr increase during alteration. To change ⁸⁷Sr/⁸⁶Sr from 0.7028 to 0.7073 and (²³⁰Th/²³²Th) from 1.25 to 1.5 would require about 14% of the Sr and 17% of the ²³⁰Th to come from the alteration component. Similar processes have been postulated to occur at other localities (for example, Kiluea³⁴ and Iceland³⁵). Furthermore, the frequent explosive magmatism, the observed release of large quantities of gas during recent eruptions, and the presence of scoriaceous spatter cones at the summit suggest that Macdonald is presently erupting highly gas-charged lavas. This implies a large increase of gas pressure over a short time interval to permit rapid gas build-up and is consistent with the effective sealing of chamber-to-surface magma conduits by seawater circulation through the crust between eruptions. □

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Neuronal correlates of a perceptual decision

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THE relationship between neuronal activity and psychophysical judgement has long been of interest to students of sensory processing. Previous analyses of this problem have compared the performance of human or animal observers in detection or discrimination tasks with the signals carried by individual neurons, but have been hampered because neuronal and perceptual data were not obtained at the same time and under the same conditions^{1–4}. We have now measured the performance of monkeys and of visual cortical neurons while the animals performed a psychophysical task well matched to the properties of the neurons under study. Here we report that the reliability and sensitivity of most neurons on this task equalled or exceeded that of the monkeys. We therefore suggest that under our conditions, psychophysical judgements could be based on the activity of a relatively small number of neurons.

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Our general methods for monitoring unit activity and eye position in alert, behaving monkeys are derived from those devised by Wurtz *et al.*⁵ and our psychophysical methods were based on those described by Newsome and Paré⁶. In brief, animals were trained to report the direction of motion of a random dot display in which some dots moved coherently while the remainder moved at random. We varied the strength of the motion signal by varying the proportion of the dots moving coherently: at 0% correlation, all the motion was random; at 100% correlation, all the motion was coherent. Near threshold, the stimulus resembled the dynamic noise seen on a domestic television set tuned between stations, combined with a barely perceptible sensation of global motion. We recorded single-neuron activity from area MT (V5), a region of the extrastriate visual cortex concerned with motion processing, where most neurons respond optimally to visual stimuli of a particular direction and speed of motion⁷⁻¹⁰. Because efficient extraction of motion signals from this stimulus requires considerable integration over space, it seemed likely that neurons in MT, which have relatively large receptive fields, would be particularly suited to this task. Newsome and Paré⁶ have recently shown that lesions of MT elevate perceptual thresholds for this task.

We used a two-alternative forced-choice procedure to measure thresholds. We placed our stimulus so that it just covered the receptive field of the neuron under study, and adjusted the speed to match that preferred by the neuron. Motion was presented either in the neuron's preferred direction or in the 'null' direction 180° away. On an individual trial, the monkey was required to hold fixation for 2 seconds while the motion stimulus was presented. At the end of the trial, the monkey indicated his judgment by transferring his gaze to one of two small light-emitting diodes, corresponding to the preferred or null direction of motion. We presented at least 30 trials (15 in each direction) for each of several correlation values chosen to elicit performance that varied from chance to near perfection, and compiled these data into psychometric functions. Recalling that performance would be 50% correct by chance, we defined the threshold as the correlation required for the monkey to judge the direction of motion correctly on 82% of the trials.

While measuring the psychophysical threshold, we recorded the activity of the MT neuron for which the stimulus parameters were optimized. The computer counted the action potentials elicited on each trial, and compiled distributions like those shown for a typical neuron in Fig. 1a. In these distributions, filled bars represent trials in which the motion was in the null direction, and cross-hatched bars indicate trials for the preferred direction. It is evident that at a correlation of 0.8% the two distributions were not different, whereas at a correlation of 12.8%, where the neuron was strongly direction-selective, they barely overlapped. To compare these neuronal data with the psychophysical data, we postulated that performance depended on a comparison between the activity of two neurons, the one under study and another differing only in that it preferred the opposite direction of motion. Under this assumption, we could use the distributions in Fig. 1a to represent the responses of the neuron under study and its 'antineuron'; we simply reversed the preferred and null directions for the antineuron. On any individual trial, therefore, the observer would compare a response drawn from the distribution represented by the hatched bars in Fig. 1a with one drawn from the distribution represented by the solid bars. The direction chosen would be the preferred direction of the neuron giving the larger response. The performance of an MT neuron could then be characterized as the probability that a randomly selected response from the hatched distribution in Fig. 1a was larger than a randomly selected response from the solid distribution. We chose this method for analysing physiological data because it most directly related neuronal performance to the directional discrimination task that the monkey was engaged in.

For the data in Fig. 1a, at a correlation of 0.8%, this decision

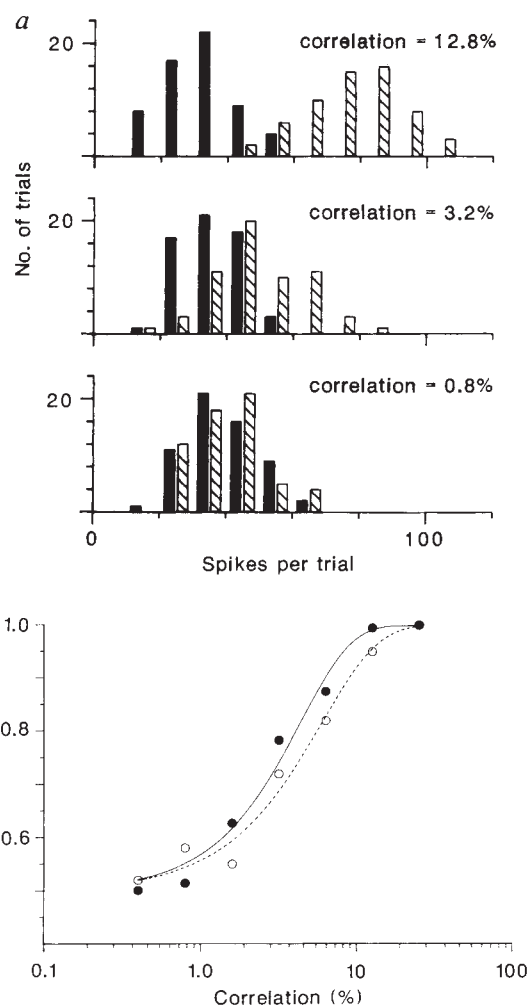


FIG. 1 Physiological and psychophysical data obtained simultaneously from a rhesus monkey. *a*, The responses of a directionally selective MT neuron at three different motion correlations spanning physiological threshold. The hatched bars represent responses to motion in the neuron's preferred direction; the solid bars indicate responses to motion in the null direction (180° opposite to the preferred). Sixty trials were performed in each direction for each of the three correlation levels. Response distributions for a range of correlation levels were used to compute a 'neurometric' function that characterized the neuron's sensitivity to the motion signal and could be compared with the psychometric function computed from the monkey's behavioural responses. *b*, Comparison of simultaneously recorded psychometric and neurometric functions. Psychophysical performance of the monkey, ○; performance of the neuron, ●. Psychophysical performance at each correlation is given by the proportion of trials on which the monkey correctly identified the direction of motion. Neuronal performance is calculated from distributions of responses like those in Fig. 1a, using a signal-detection method described in the text. The physiological and psychophysical data form similar curves, but the data for the neuron lie to the left of the data for the monkey, meaning that the neuron was somewhat more sensitive than the monkey. We fit the data with smooth functions of the form introduced to psychophysics by Quick¹². Threshold, defined as the correlation for which the direction of motion was identified correctly on 82% of the trials, was 6.1% for the monkey and 4.4% for the neuron.

rule chose the correct direction only on about half the trials (random performance), whereas at a correlation of 12.8% it performed nearly perfectly. We used a method based on signal detection theory¹¹ to estimate this choice probability for each correlation value, and plotted the results as 'neurometric functions' formally equivalent to the psychometric functions representing the psychophysical data^{2,4}. The two functions for this example neuron are shown in Fig. 1b; filled circles represent neurometric data, open circles represent psychometric data. Evidently the two curves are very similar, with the neurometric

data points lying slightly to the left of the psychometric data points; in this case the neuronal threshold was slightly lower than the psychophysical one. We used a likelihood-ratio statistic to test the hypothesis that the psychometric and neurometric functions were the same. For this neuron, this hypothesis could not be rejected ($P > 0.05$).

We performed this analysis for 45 neurons recorded from one monkey, and 15 neurons from a second. Figure 2 shows a histogram of the distribution of the ratio of neurometric to psychometric thresholds for these 60 neurons. Values of this ratio of <1 represent cases where the neuron's threshold was lower than the monkey's; values >1 represent cases where the monkey's performance was better than the neuron's. Intuitively, it might be expected that the behavioural threshold would be lower than any particular neuronal threshold but, in most cases, neuronal thresholds and perceptual thresholds were similar. Indeed in some cases, neuronal thresholds were substantially lower than perceptual thresholds. For 20 of the 60 neurons in our sample, the psychometric and neurometric functions were statistically indistinguishable ($P > 0.05$); in 18 of the 40 remaining cases, neuronal thresholds were lower than perceptual thresholds. In other words, if the monkeys were able to select and measure the discharge of some of these neurons as we did, their performance could have been better than it actually was.

An inability to select the most informative signals can be considered as a kind of perceptual uncertainty, of the kind modelled by Pelli¹³. Obligated to monitor signals from many sources less informative than the one perfectly tuned to the visual target, the animal's perceptual performance would be degraded, because each sub-optimal source would contribute more noise than signal. Neuronal performance would then exceed psychophysical performance. Our results suggest, however, that this effect is not large. Substantial uncertainty would make the psychometric function steeper than the neurometric function¹³, but as was the case for the example shown in Fig. 1b, the slopes of these two functions are usually similar. We thus conclude that under our conditions, the monkey's perceptual decision is not greatly affected by irrelevant signals introduced by uncertainty.

The apparent absence of uncertainty leads, however, to another question: if a perceptual decision can be based with relative certainty on the discharge of the most informative neurons, why is behavioural performance not further enhanced by using a pooled signal derived from many such informative neurons? If enough such neurons were present, such pooling

would substantially improve psychophysical performance by averaging out the noise that obscures weak signals. Our data show that in most cases, the neuronal and psychophysical performances are similar, indicating that signals from many neuronal sources are not pooled to reduce perceptual thresholds.

One way to account for the absence of either pooling or uncertainty effects is to suggest that the variability in the responses of similarly tuned neurons is correlated. Both pooling and uncertainty act as we have stated only if different neuronal signals are perturbed by independent sources of variation. If the sources are not independent, then uncertainty does no damage and pooling provides no benefit, because different neurons are carrying similar signals. The rich network of shared connections that link MT neurons with the retina might well produce correlation among neurons with related selectivities, but this possibility has not been studied. Our lack of information about the degree of shared variability makes it impossible for us to assert that the neurons whose responses we have recorded are the ones that contribute to the monkey's perceptual judgements. Nonetheless, our results show that a reasonable account of the monkey's performance can be constructed, using a simple decision rule, from signals carried by small numbers of neurons whose selectivities are well matched to the demands of the perceptual task. □

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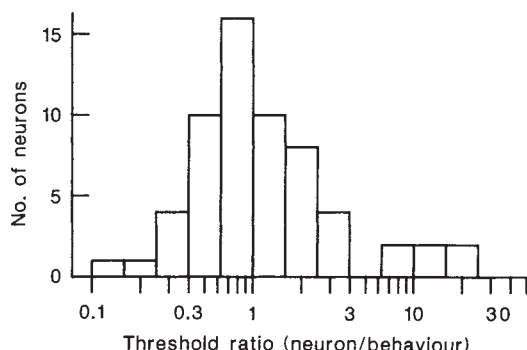


FIG. 2 Comparison of psychophysical and physiological thresholds obtained for 60 MT neurons in two rhesus monkeys. The frequency histogram shows the distribution of the ratio of the physiological threshold to the psychophysical threshold for all the neurons for which we obtained data. A value of 1 represents perfect correspondence between psychophysical and physiological thresholds; values <1 indicate that the physiological threshold was lower than the psychophysical threshold, whereas values >1 indicate the converse. The directional preferences of the 60 neurons were roughly uniformly distributed, and there was no reliable association between a neuron's direction or speed preference and its threshold relative to the perceptual threshold.

Hippocampal abnormalities in amnesic patients revealed by high-resolution magnetic resonance imaging

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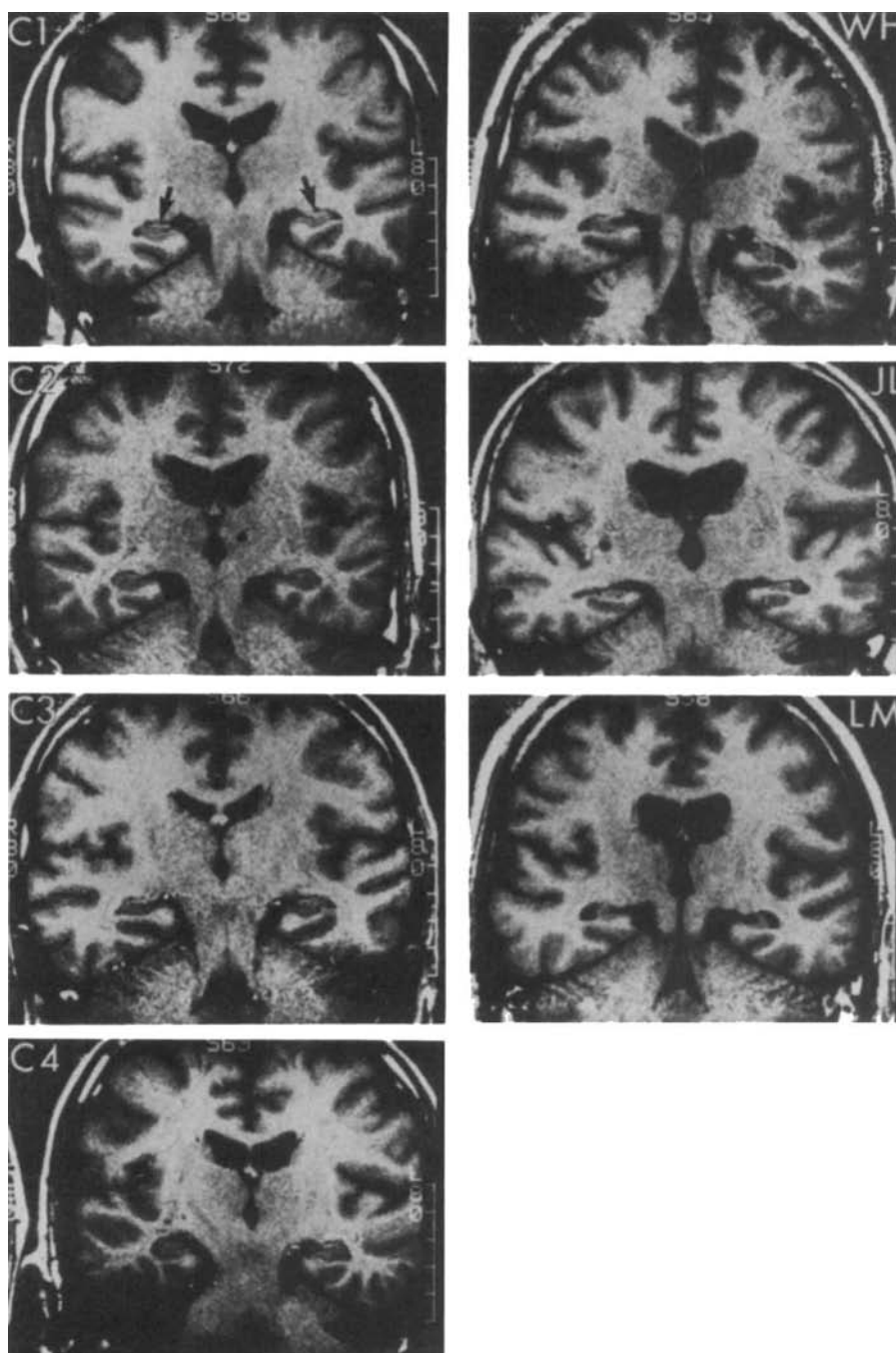
THE identification of brain structures and connections involved in memory functions has depended largely on clinico-pathological studies of memory-impaired patients^{1–4}, and more recently on studies of a primate model of human amnesia^{5,6}. But quantitative neurobehavioural data and detailed neuropathological information are rarely available for the same patients^{7–9}. One case has demonstrated that selective bilateral damage to the hippocampus causes a circumscribed memory impairment in the absence of other intellectual deficits⁹. This finding, in conjunction with evidence from humans^{10,11} and monkeys^{12–16}, indicates that the hippocampus together with adjacent and anatomically related structures is essential for the formation of long-term memory, perhaps by virtue of

TABLE 1 Subject characteristics

Patient	Age (yr)	WAIS-R	WMS-R				
			Attention/conc.	Verbal	Visual	General	Delay
Controls							
1	65	103	92	96	109	102	102
2	70	110	111	91	93	89	100
3	57	119	114	114	120	121	138
4	63	137	124	125	123	129	138
Mean	63.8	117.3	110.3	106.5	111.3	110.3	119.5
Amnesics							
W.H.	66	113	88	72	82	67	<50
J.L.	69	116	122	73	83	74	58
L.M.	58	111	132	87	96	90	65
Mean	64.3	113.3	114.0	77.3	87.0	77.0	57.7

WAIS-R, Wechsler Adult Intelligence Scale-Revised; WMR-R, Wechsler Memory Scale-Revised. The WAIS-R and each of the five indices of the WMS-R yield a mean score of 100 in the normal population with a standard deviation of 15. The WMS-R does not provide numerical scores for subjects who score below 50. Therefore, the single value below 50 was scored as 50 for computing a group mean.

FIG. 1 T1-weighted (TE=400 ms, TR=20 ms) images of 5-mm thick slices through the hippocampal formation. These photographs show the first or second of the three levels through the hippocampal formation that were submitted to quantitative analysis. Panels on the left (C1-C4) show images from neurologically intact control subjects, and panels on the right show images from three amnesic patients (W.H., J.L. and L.M.). The area of the hippocampal formation (indicated by arrows in panel C1) in the amnesic patients averaged 49% of the corresponding area in control subjects. In contrast, the average area of the temporal lobe (measured from the fundus of the collateral sulcus to the inferior limiting sulcus) was nearly identical in the two groups. The parahippocampal gyrus is smaller in J.L. than in the other amnesic and control subjects. The photographs show the left side of the brain on the right side of each panel. The calibration bar at the right of each panel represents 5 cm in 1-cm increments.



the extensive reciprocal connections between the hippocampal formation and putative memory storage sites in the neocortex¹⁷. Although cognitive studies of amnesia provide useful information about the functional organization of normal memory^{1,18-21}, it has not usually been possible to relate memory impairment to anatomy in living patients. We have developed a high-resolution protocol for imaging the human hippocampus with magnetic resonance that permits visualization of the hippocampal formation in substantial cytoarchitectonic detail, revealing abnormalities in patients with severe and selective memory impairment.

Three male amnesic patients and four male control subjects were examined (Table 1). Patient W.H. became amnesic rapidly over the course of several days in 1986 without antecedent head trauma, seizure or unconsciousness. Patient J.L. became amnesic gradually during a period of about two years (from early 1985 to early 1987); his cognitive status has remained stable since that time. The aetiology in these two cases was unknown. Patient L.M. became amnesic in 1984 as the result of a respiratory arrest which occurred during an epileptic seizure. All three patients were severely impaired on standard tests of verbal and non-verbal memory^{22,23}. For example, learning of 10 noun pairs across three trials²⁴ was 0.3, 0.3 and 1.0 pair (controls; 6.3, 7.5 and 8.5). The amnesic patients performed well on other cognitive tests, such as on the non-memory portions of the Dementia Rating Scale²⁵ (maximum score, 119; patients, 116.3; controls, 118.3) and on the Controlled Word Association Test (FAS)²⁶ (patients, 52.0 words; controls, 41.8 words).

Magnetic resonance examinations were performed with a 1.5-tesla superconducting magnet (General Electric, Milwaukee). In preliminary studies, we determined that the optimal resolution of the hippocampus was obtained when the hippocampus was imaged perpendicular to its long axis. Accordingly, each subject was supine on the magnetic resonance examination table within the magnet, and the head was tilted posteriorly so that the line joining the lips and the external auditory canal was perpendicular to the table (and the longitudinal axis of the magnetic field). A sagittal, T1-weighted (TR = 200 ms, TE = 20 ms, 1 excitation (NEX)) spin-echo sequence (field of view (FOV) = 24 cm, matrix = 256 × 128) centred at the mid-line showed the long axis of the hippocampus clearly. The head of the patient was then repositioned slightly if necessary to orientate the hippocampus appropriately. Coronal images were then obtained using a T1-weighted sequence (TR = 400 ms, TE = 20 ms, 6 NEX; FOV = 16, matrix = 256 × 256), which provided six interleaved, 5 mm-thick sections (no interslice gaps) with 0.625 mm in-plane spatial resolution. We obtained the first section just caudal to the pes hippocampus and were able to survey 30 mm of the hippocampus out of a

total rostrocaudal length of 40 mm. Coronal proton-density and T2-weighted images (TR = 2,700 ms, TE = 30 and 80 ms, 1 or 2 NEX; FOV 16, matrix = 256 × 256) were also obtained.

The images obtained by our high-resolution, T1-weighted protocol showed the hippocampal formation in considerable detail (Figs 1 and 2). For example, Fig. 2a illustrates in a normal subject the pyramidal cell fields of the subiculum and hippocampus, the fimbria, perforant path, stratum lacunosum-moleculare, the alveus, and the molecular layer of the dentate gyrus. To calculate areal boundaries within the temporal lobes, we selected slices that were located at about the same rostrocaudal level in each subject. For each series of images, the first section caudal to the pes hippocampus and the two adjacent caudal sections were selected for analysis.

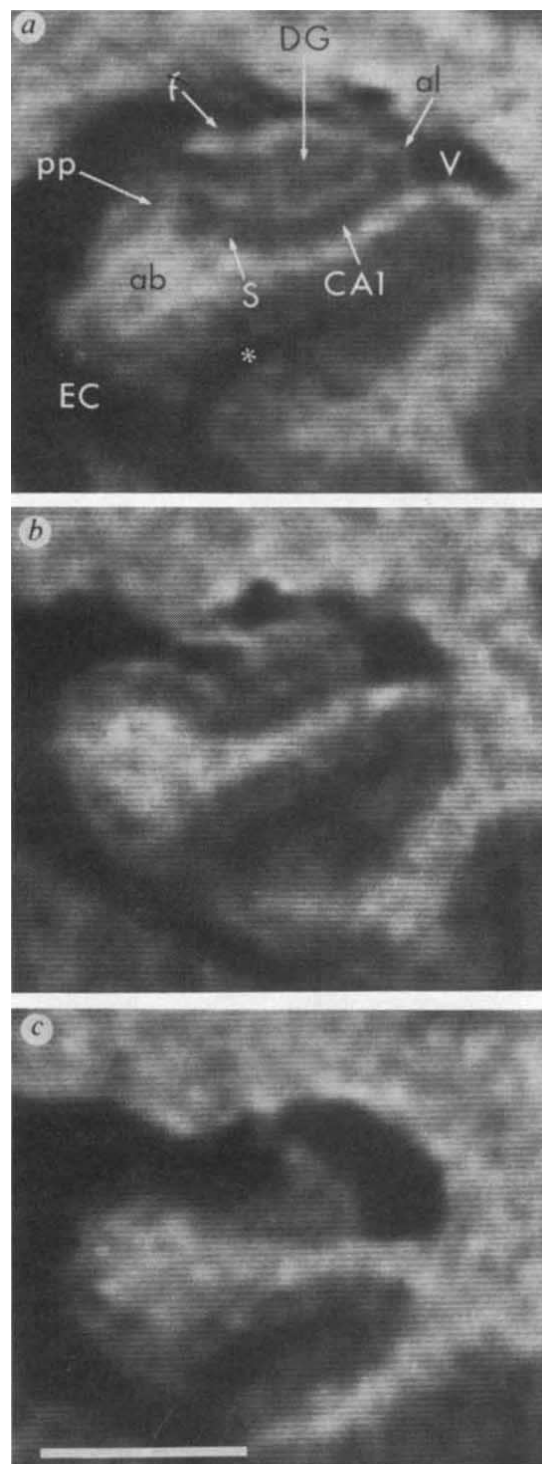


FIG. 2 Higher magnification magnetic resonance images than those shown in Fig. 1 of the left hippocampal formation. **a**, T1-weighted image (TE = 400 ms, TR = 20 ms) of a 5-mm thick section through the hippocampal formation of a 24-year-old normal subject. It is possible to distinguish the angular bundle (ab), the perforant path (pp), the alveus (al), the molecular layer of the dentate gyrus (DG), and the fimbria (f). The pyramidal cell layer of the subiculum (S) and the CA1 field of the hippocampus (which are ~1.5-mm thick in the coronal plane at this level) can also be seen. The entorhinal cortex (EC) lies just ventral to the angular bundle. The region of high signal intensity superficial to the pyramidal cell layer marks the stratum lacunosum-moleculare, in which the perforant path projects to the hippocampus and dentate gyrus. The temporal horn of the lateral ventricle (V) lies just lateral and dorsal to the hippocampus, and the asterisk marks the position of the collateral sulcus. **b**, Hippocampal formation of control subject C1 at the same level seen in Fig. 2. Whereas this image is not as distinct as the one from the younger subject, most of the anatomical features identified in that image can also be distinguished here. **c**, Hippocampal formation of amnesic patient L.M. at the same level as in Fig. 1. In contrast to the images in **a** and **b**, the fimbria is not distinct in this image, and it is difficult to determine the boundary of the pyramidal cell layer in the subiculum or hippocampus. In addition, there is marked reduction of the size of the dentate gyrus, hippocampus, and subiculum. The calibration bar represents 1 cm in all three panels.

Using 11 × 14 inch photographs and a microcomputer-linked digitizing tablet, the area of the hippocampal formation (defined as the fimbria, dentate gyrus, hippocampus proper and subiculum) was computed on each side and in each of the three sections. We then measured the combined area of the hippocampal formation and the parahippocampal gyrus (extending from the subiculum to the fundus of the collateral sulcus) and the area of each temporal lobe (minus the hippocampal formation and parahippocampal gyrus). The outline for the temporal lobe measurement extended from the fundus of the collateral sulcus to the inferior limiting sulcus of the insular cortex. The left and right lateral ventricles and the third ventricle were also measured. We expressed the area of each of the regions as the average per section of the areal values for the sampled sections.

The area of the temporal lobe was nearly identical in the two groups (controls: left temporal lobe, 11.57 cm², right temporal lobe, 12.75 cm²; patients: left temporal lobe, 11.85 cm², right temporal lobe, 13.57 cm²). By contrast, the hippocampal formation (HF) in the patients was markedly reduced in size (controls: left HF, 0.60 cm², right HF, 0.67 cm²; patients: left HF, 0.29 cm², right HF, 0.33 cm²). The area of the hippocampal formation in the patients was 49% of that in the control subjects ($t_s = 5.4$, $P < 0.01$). The results were the same when the area of the hippocampal formation was calculated as a percentage of the size of the temporal lobe (controls, 5.3%, patients, 2.5%). The lateral ventricles (patients, 4.31 cm²; controls, 2.84 cm²) and the third ventricle (patients, 1.05 cm²; controls, 0.76 cm²) were somewhat larger in the patients than in the controls, but these differences were not significant ($0.1 > P < 0.05$). Patient J.L. had left and right ventricles outside the normal range; patients J.L. and L.M. had third ventricles outside the normal range. Finally, the area of the parahippocampal gyrus was smaller in patient J.L. (the average area of each side was 1.01 cm², or 7.5% of the area of the temporal lobe). The parahippocampal area was nearly the same in the other two amnesic patients as in the control subjects (patients: 1.29 cm², 10.5% of the temporal lobe area; controls: 1.28 cm², 10.5% of the temporal lobe area).

T2-weighted images revealed no regions of abnormal signal intensity in the brain of patient L.M. The other two patients had non-specific, abnormal, hyper-intense foci in the striatum, as previously reported; for example, in ischaemia, de-myelination, or infarction²⁷. Patient J.L. had several additional hyper-intense foci distributed in the white matter.

This high-resolution magnetic resonance protocol focused on one area of interest and did not survey all brain regions. Accordingly, we cannot exclude the possibility that additional abnormalities might have been detected if other areas had been surveyed as carefully as the hippocampal formation. Nevertheless, circumscribed memory impairment has been associated previously with histologically confirmed hippocampal lesions in the absence of other significant damage⁹, so the abnormalities that we have identified are likely to have contributed substantially to memory dysfunction in all three patients.

Our magnetic resonance protocol has acquired images with a better resolution of the hippocampal formation than is obtained by more standard protocols²⁸⁻³⁰. We attribute this improved resolution to the fact that we obtained images precisely perpendicular to the long axis of the hippocampus. Because damage to the hippocampal formation occurs prominently in Alzheimer's disease³¹, and memory impairment is one of its earliest symptoms, this protocol might be useful in achieving an early diagnosis of the disease. □

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Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*

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THE primary event in the pathogenesis of severe malaria in *Plasmodium falciparum* infection is thought to be adherence of trophozoite- and schizont-infected erythrocytes to capillary endothelium¹, a process called sequestration. Identifying the endothelial molecules used as receptors is an essential step in understanding this disease process. Recent work implicates the membrane glycoprotein CD36 (platelet glycoprotein IV; refs 2-5) and the multi-functional glycoprotein thrombospondin⁶ as receptors. Although CD36 has a widespread distribution on microvascular endothelium⁷, it may not be expressed on all capillary beds where sequestration occurs, especially in the brain⁸. The role of thrombospondin in cell adhesion, *in vitro* or *in vivo*, is less certain^{4,9}. We have noticed that some parasites bind to human umbilical-vein endothelial cells independently of CD36 or thrombospondin. To screen for alternative receptors, we have developed a novel cell-adhesion assay using transfected COS cells, which confirms that CD36 is a cell-adhesion receptor. In addition, we find that an endothelial-binding line of *P. falciparum* binds to COS cells transfected with a complementary DNA encoding intercellular adhesion molecule-1. As this molecule is widely distributed on capillaries and is inducible¹⁰, this finding may be relevant to the pathogenesis of severe malaria.

We have observed that binding of red blood cells infected with Gambian wild isolates of *P. falciparum*, in the C32 amelanotic melanoma cell adherence assay varies markedly, but does not correlate with severity of clinical malaria¹¹. More

recently we have found that there is no correlation between the capacity of individual isolates to bind to C32 cells and endothelial cells (unpublished observations). This also applied to laboratory lines. The cloned parasite line FCR3A2 showed strong CD36-dependent binding to C32 cells but did not bind to endothelial cells, whereas the selected line ITO4, derived from the Brazilian IT isolate, bound to both cell types (Fig. 1). Adhesion of ITO4 infected cells to endothelial cells is not blocked by a range of monoclonal antibodies to CD36 or by a polyclonal anti-thrombospondin antibody (data not shown).

It seemed possible that an additional endothelial receptor could explain these discrepancies and we therefore devised a novel adhesion assay using transiently transfected COS cells¹² as a means of screening for such a receptor.

A cDNA clone coding for CD36 was isolated from a placental cDNA library by transient expression and panning¹². We first

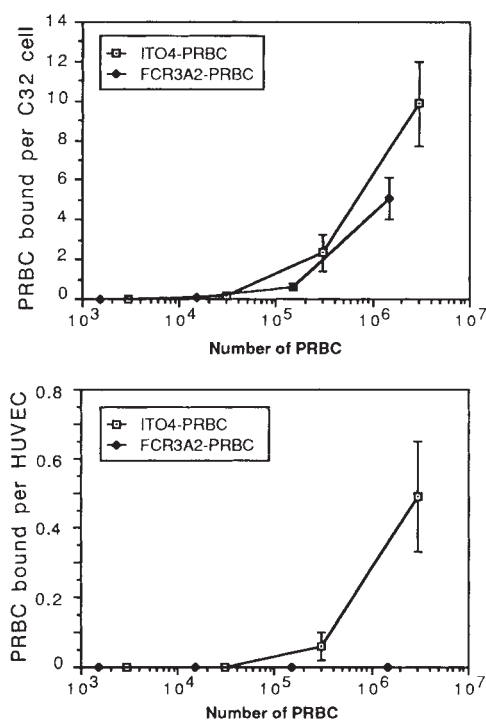


FIG. 1 Comparison of endothelial and melanoma binding of the parasite lines FCR3A2 and ITO4. Binding of parasite-infected red blood cells (PRBC) to C32 melanoma cells (top) or endothelial cells (bottom) is shown for the FCR3A2 clone (closed diamonds) and the ITO4 line (open squares). Points show the means $\pm$ 1 s.e.m. of triplicate coverslips. Representative data from a single experiment are shown.

METHODS. Parasites were cultured as previously described^{24,25} in 96% N₂, 1% O₂, 3% CO₂ and used in binding experiments at mature trophozoite stage at 2% haematocrit, 6% parasitaemia (ITO4) or 3% parasitaemia (FCR3A2). C32 amelanotic melanoma cells were cultured in RPMI 1640 medium, supplemented with penicillin, streptomycin, glutamine and 10% FCS (Gibco) (R10 medium). Human umbilical vein endothelial cells (HUVEC) were isolated and cultured as previously described²⁶ with minor modifications. Contaminating cord blood cells were removed by centrifugation on 90% Lymphoprep (Nycoprep) in serum-free medium at 1,500g for 15 min. Endothelial cells and adherent lymphocytes are the only cells retained at the interface (A.R.B., unpublished data). C32 cells were detached with trypsin-EDTA and seeded onto Thermanox 13 mm coverslips at a density of 5×10^3 per coverslip; HUVEC were seeded direct as primary cultures at 10^4 per coverslip. After 48 h they were washed in serum-free medium, fixed for 45 min in 1% formaldehyde in PBS, washed three times in binding medium, and used in the binding assay. Bindings were carried out as previously described for 1 h, gently agitating the coverslips every 10 min. Coverslips were then washed for 1 h by inversion in fresh binding medium, fixed in 1% glutaraldehyde in binding medium, stained in 5% Giemsa for 20 min, dried, mounted in DPX mountant and counted. Counting was performed blind; 200 melanomas or 300 endothelial cells were counted and the binding expressed as number PRBC bound per target cell.

confirmed that transiently expressed CD36 behaved as a receptor for FCR3A2- and ITO4-infected cells. Both lines bound to CD36-transfectants (Fig. 2c, d) but not to resting or mock-transfected COS cells (Fig. 2a, b), nor to COS cells transfected with cDNAs encoding CD2 or CD16 (data not shown). Expression of the cDNA by the transfectants was confirmed using appropriate monoclonal antibodies in indirect immunofluorescence assays (data not shown). C32-non-binding parasites, such as T9/96, did not bind to CD36-transfectants.

While screening for other cell-adhesion receptors, we found that as well as binding to CD36-transfectants, erythrocytes infected with ITO4 also bound to COS cells transfected with a cDNA encoding the protein intercellular adhesion molecule-1 (ICAM-1; ref. 13; see Fig. 2f). FCR3A2-infected cells did not bind at all to ICAM-1 transfectants (Fig. 2e).

The anti-ICAM-1 monoclonal antibody 7F7 (ref. 14) and the anti-CD36 monoclonal antibody IVC7 (ref. 15) were used in binding inhibition experiments. Each antibody reacted only with the appropriate transfectant in immunofluorescence and immunoprecipitation experiments (data not shown). Each antibody blocked adhesion only to the appropriate transfectant (Fig. 3, top). These results exclude either contamination of ICAM-1-

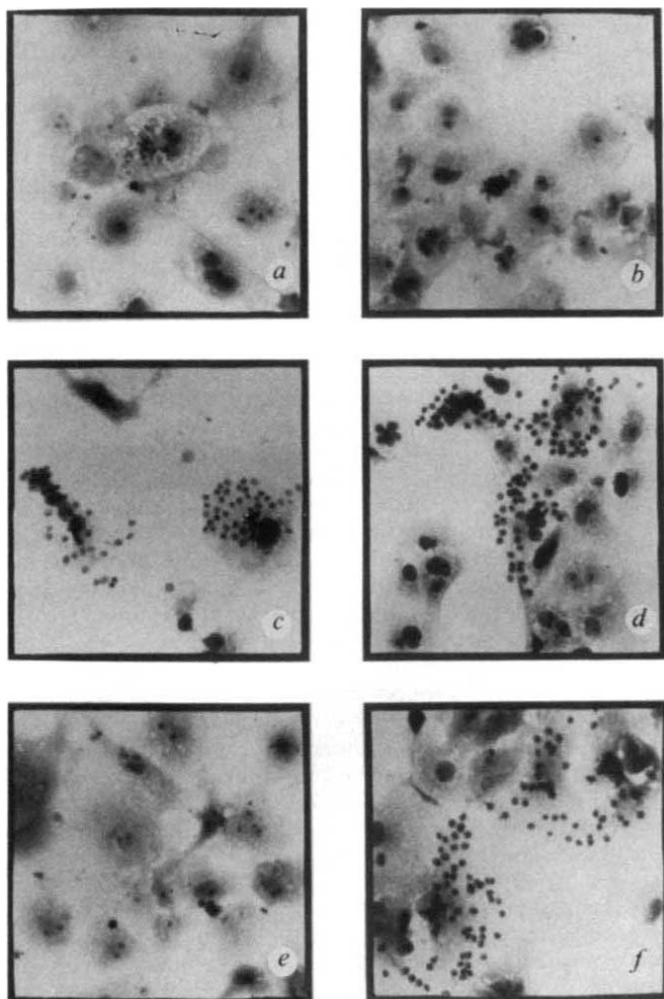


FIG. 2 Binding of PRBC to transiently transfected COS cells. Target cells are mock-transfected COS cells (a, b), CD36-transfectants (c, d) or ICAM-1-transfectants (e, f). Erythrocytes infected with FCR3A2 (a, c, e) or ITO4 (b, d, f) were used in the binding assay.

METHODS. COS cells were transfected with cDNA molecules cloned in the CDM7 expression vector as previously described¹². After transfection (24 h) the cells were detached with trypsin-EDTA, seeded onto 13 mm Thermanox coverslips at a density of 10^4 cells per coverslip and cultured in R10 medium. After 24–48 h, coverslips were washed in serum-free medium and fixed for 45 min in 1% formaldehyde in PBS. Binding assays were performed as above.

cDNA with CD36-cDNA or a cross-reacting epitope mediating binding. The control monoclonal antibody W6/32, which recognizes a monomorphic HLA determinant on both human and COS cells, had no effect on binding.

When the monoclonal antibodies were tested on cultured endothelium, the binding of ITO4 was significantly inhibited by anti-ICAM-1 antibody, but little affected by anti-CD36 antibody (Fig. 3, bottom). The converse applied to ITO4 binding to C32 melanoma cells. The ITO4 line thus principally uses CD36 as a receptor on C32 cells, but binds to ICAM-1 on cultured endothelium. By contrast, FCR3A2 binds in a CD36-dependent manner to C32 cells and transfected COS cells, but cannot bind to ICAM-1 transfectants or to endothelium. The ability to bind to ICAM-1 is therefore not a universal feature of all binding parasite lines. It has previously been noted that parasite binding to cultured endothelium is consistently lower than binding to C32 cells^{16,17}. These data could now be explained by the use of different receptors, and a variable degree of ICAM-1 expression in different endothelial cultures.

ICAM-1, a variably glycosylated glycoprotein, is a ligand for the leucocyte integrin LFA-1 (refs 13, 18). It permits antigen-independent interactions between cells of the immune system and seems to play a central role in the generation of the immune response^{14,19,20}. It is also widely distributed on capillary endothelium, where it may be involved in lymphocyte adhesion¹⁰. Levels of ICAM-1 can be up-regulated both *in vitro* and *in vivo* on endothelial cells in response to tumour necrosis

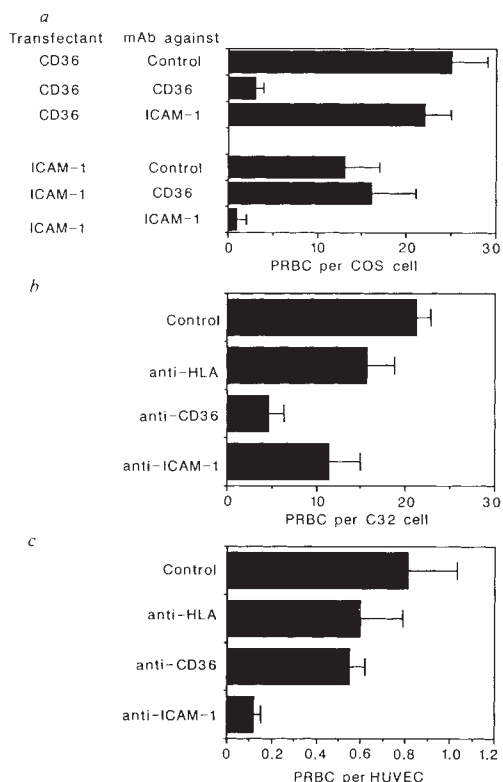


FIG. 3 Effect of monoclonal antibodies on binding of ITO4-infected erythrocytes to transfected COS cells (a), C32 cells (b) and HUVEC (c). Monoclonal antibodies: 7F7 (anti-ICAM-1); IVC7 (anti-CD36); W6/32 (anti-HLA as control antibody). Bars, labelled control, no antibody included. Points are means ± 1 s.e.m. of triplicate coverslips. Representative data from a single experiment are shown.

METHODS. Target cells were prepared as for Figs 1 and 2, and used in binding assays as above. Antibody was diluted 1:100 in binding medium (ascites exudate, IVC7 and 7F7) or 1:20 (culture supernatant, W6/32) and incubated with fixed coverslips at room temperature for 60 min. Coverslips were dip-washed in binding medium three times to remove unbound antibody, and used in the binding assay as above. Binding to transfectants was quantitated by counting 300 COS cells from blinded coverslips and deriving the mean number of PRBC bound per binding COS cell.

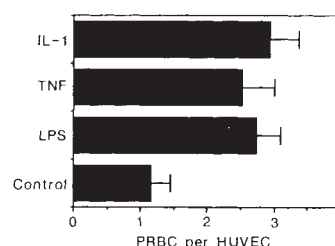


FIG. 4 Binding of ITO4-infected erythrocytes to resting and stimulated endothelium. Points are means ± 1 s.e.m. of triplicate coverslips. Representative data from a single experiment are shown.

METHODS. Secondary cultures of endothelium were detached and seeded onto coverslips as above. After 48 h they were washed and the medium replaced with fresh growth medium containing no mediator (control), recombinant human tumour-necrosis factor (TNF) at 200 U ml⁻¹, recombinant human interleukin-1 (IL-1) at 50 U ml⁻¹, or bacterial lipopolysaccharide (LPS) at 1 μ g ml⁻¹. After 12 h the coverslips were washed and used immediately in the binding assay as above.

factor (TNF), interleukin-1, and γ -interferon^{21,22}. We therefore examined the binding of ITO4-infected cells to cytokine and endotoxin-stimulated endothelium and found increased levels of binding after stimulation (Fig. 4).

In acute malaria, increased levels of cytokines, notably TNF, have been detected and seem to correlate with disease severity (ref. 23 and D. Kwiatkowski, personal communication). The identification of ICAM-1 as an additional cell-adhesion receptor raises the possibility that cases of severe malaria occur when parasites that can bind strongly to ICAM-1 coincidentally infect individuals with high endothelial levels of ICAM-1. Sequestration would also occur in those capillary beds where CD36 is expressed, so that the final clinical outcome would reflect the balance between the parasite-determined affinities for different receptors and the host-specific levels of these receptors. Further studies are in progress to address these questions.

Note added in proof: The sequence of the CD36 cDNA clone described in this paper is identical to that recently reported by Oquendo *et al.*²⁷. We have found no significant homologies between CD36, ICAM-1 and thrombospondin.

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Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells

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T LYMPHOCYTES are found not only as recirculating cells in the lymphoid system, but also as immobile cells in certain epithelia. T-cell antigen receptors (TCR) of both α/β and γ/δ -heterodimer subtypes can exhibit an extremely high degree of diversity. The diversity of α/β TCRs derives from the use of a large number of variable (V) gene segments, as well as junctional diversity generated during rearrangement of these segments, whereas the diversity of γ/δ TCRs derives largely from junctional elements, with a smaller contribution from a limited number of V gene segments¹. Many T cells in the epidermal and intestinal epithelia of mice express TCR composed of γ/δ heterodimers^{2-5,14,15}. We demonstrate here that γ/δ TCRs of T cells in both these tissues are restricted in V gene usage, with different elements predominating. The TCR junctional diversity of epidermal T cells, however, is extremely limited, whereas that of intestinal T cells is extremely diverse. The distinctive features of these two populations suggest

that they develop or are selected differently for particular tissue-specific functions.

Uncultured polyclonal dendritic epidermal cell (dEC) populations in most mouse strains predominantly use $V_{\gamma 3}$ and $V_{\delta 1}$ gene segments⁶⁻⁹. We examined rearrangements of these segments in a polyclonal population of dEC-enriched epidermal cells. As shown in Fig. 1, the junctions of 18 out of 21 potentially productive $V_{\gamma 3}$ - $J_{\gamma 1}$ rearrangements were identical, as were the junctions of 12 out of 15 $V_{\delta 1}$ - $J_{\delta 2}$ rearrangements. These predominant $V_{\gamma 3}$ - $J_{\gamma 1}$ and $V_{\delta 1}$ - $J_{\delta 2}$ rearrangements were also identical to those previously found in each of five independently derived dEC clones⁶. In contrast, no one sequence predominated in the dEC $V_{\delta 1}$ - $J_{\delta 1}$ rearrangements. In the predominant $V_{\gamma 3}$ and $V_{\delta 1}$ sequences, as well as in most of the other dEC TCR rearrangements, non-germline encoded nucleotides (NGE, or N-regions¹⁰⁻¹¹) were absent, although a few contained one or two NGE bases. These data thus support the conclusion that the TCR repertoire of dEC is severely limited. In addition, the use of $V_{\gamma 3}$ and $V_{\delta 1}$ gene segments, the minimal amount of NGE insertion, and the use of $D_{\delta 2}$ alone in these rearrangements are also characteristic of the rearrangements found early in fetal development^{12,13}, supporting the model of an early fetal origin of dEC^{6,7}.

Another epithelium-associated T cell is the intestinal intraepithelial lymphocyte (IEL)¹⁴⁻¹⁸. Previous studies^{14,15,18} and our results (not shown) suggest that the majority of IEL γ chains are encoded by the $V_{\gamma 5}$ gene. Polymerase chain reaction (PCR) products of predicted sizes were obtained when IEL DNA was amplified with the $J_{\gamma 1}$ primer and either $V_{\gamma 2}$, $V_{\gamma 4}$, or $V_{\gamma 5}$ (refs 13, 20) primers, but no product was obtained with $V_{\gamma 3}$ or $V_{\gamma 1}$ primers (data not shown).

Only one of twelve IEL $V_{\gamma 2}$ subclones represented a productive rearrangement (Fig. 2a). One single sequence accounted for 9 out of 11 productive $V_{\gamma 4}$ rearrangements, and a second

a					
	V	N	J		
Germline Vy3	TGTGCCTGCTGGGATCT				
Germline Jy1			ATAGCTCAGGTTTT		
3.2.2	**TGTGCCTGCTGGG		ATAGCTCAGGTTTT	+	(18)
3.2.4	TGTGCCTGCTGG	A	ATAGCTCAGGTTTT	+	
3.2.18	TGTGCCTGCTGGGATC		GCTCAGGTTTT	+	
3.2.20	TGTGCCTGCTGGG	C	TAGCTCAGGTTTT	+	
3.2.16	TGTGCCTGCTGGGAT		ATAGCTCAGGTTTT	-	(2)
3.2.1	TGTGCCTGC		ATAGCTCAGGTTTT	-	
3.2.12	TGTGCCTGCTGGG		TAGCTCAGGTTTT	-	
3.2.14	TGTGCCTGCTGGGAT		TAGCTCAGGTTTT	-	
3.2.29	TGTGCCTGCTGG	AT	ATAGCTCAGGTTTT	-	
3.2.30	TGTGCCTGCTGGGATCT		ATAGCTCAGGTTTT	-	

b						
	V	D	N	J		
GERMLINE Vδ1	TGTGGGTACAGATAT					
GERMLINE Dδ2		ATCGGAGGGATACGAG				
GERMLINE Jδ2				CTCCTGGGAC		
J2.3	**TGGGGGTACAGATAT	CGGAGGGA	G	CTCCTGGGAC	+	(12)
J2.2	TGTGGGTACAGATAT	CGGAGGGATACGA		TCCTGGGAC	+	
J2.8	TGTGGGTACAGAT	CGGAGGGATACGAG		CTCCTGGGAC	+	
J2.10	TGTGGGTACAGATAT	CGGAGGG	TG	CTCCTGGGAC	+	

c							
	V	N	D	N	J		
GERMLINE Vδ1	TGTGGGTACAGATAT						
GERMLINE Dδ2			ATCGGAGGGATACGAG				
GERMLINE Jδ1					CTACCGACAAA		
J1.2	TGTGGGTACAGATAT		CGGAGGGATACGAG		CTACCGACAAA	+	(3)
J1.1	TGTGGGTCA	T	ATCGGAGGGA		TACCGACAAA	+	
J1.11	TGTGGGTACAGATAT		CGGAGGGATACGAG		CCGACAAA	+	(2)
J1.18	TGTGGGTACAGATAT		CGGAGGGATACGAG		ACAAA	+	
J1.3	*TGTGGGTACAGATAT		CGGAGGGA		TACCGACAAA	-	(2)
J1.5	TGTGGGTACAGATA	AT	ATCGGAGGGA		TACCGACAAA	-	
J1.9	TGTGGGTACAGATAT		CGGAGGGATA		CTACCGACAAA	-	
J1.13	TGTGGGTACAGAT		CGGAGGGATACGAG		CTACCGACAAA	-	
J1.16	TGTGGGTACAGATAT		CGGAGGGAT		CCGACAAA	-	
J1.17	TGTGGGTACAG		GAGGGATACGAG		CTACCGACAAA	-	
J1.21	TGTGGGTACAGATAT		CGGAGGGATAC		CGACAAA	-	
J1.27	TGTGGGTACAGATAT		CGGAGGGAT	G	CTACCGACAAA	-	
J1.31	TGTGGGTACAGATAT		CGGAGGGATACGAG		CGACAAA	-	

FIG. 1 Junctional sequences of $V_{\gamma 3}$ and $V_{\delta 1}$ rearrangements in dEC. a, Rearrangements of $V_{\gamma 3}$ to $J_{\gamma 1}$. b, Rearrangements of $V_{\delta 1}$ to $J_{\delta 2}$. c, Rearrangements of $V_{\delta 1}$ to $J_{\delta 1}$. 'N' denotes nucleotides not present in germline sequences. Because of shared sequences at the ends of some germline gene segments, assignment of nucleotides to one segment or the other in this and other figures is sometimes arbitrary. The sequences are designated as productive (+) or non-productive (-) rearrangements as regards the reading frame of the J segment. The number of sequences presented for any one V gene segment, in this and other figures, does not necessarily indicate the relative levels of V gene segment rearrangement or expression. Asterisks mark sequences previously found in dEC clones⁶. METHODS. Epidermal cells from Balb/c mice were enriched for dEC by Ficoll-hypaque density gradient centrifugation²⁸ and genomic DNA prepared by phenol extraction and ethanol precipitation. Sequences spanning recombination junctions were amplified by PCR^{6,29} and the products digested by appropriate restriction endonucleases and gel-purified before ligation to M13mp18 or M13mp19. PCR primers used were: GCACTGGATCCAAC-TGAAAGAAG($V_{\gamma 3}$), GGGAAGCTTACCAGAGGGAATTACTATGAG($J_{\gamma 1}$), AATAG-GAATCTACTGATGGTGG($V_{\delta 1}$), GGGGAATCTTGGTCCACAGTCACCTGGG($J_{\delta 1}$) and GGGGAAGCTTGGGGCTCCACAAAGAGC($J_{\delta 2}$). Subclones containing appropriate inserts were picked at random or after screening plaques with ³²P-labelled PCR primers, and sequenced by the dideoxy chain-termination method with the Sequenase enzyme (USB).

sequence accounted for 11 of the 18 non-productive rearrangements (Fig. 2b), suggesting that only very few IEL have $V_{\gamma 4}$ rearrangements; the PCR amplification might then yield a product biased towards only a few productive or non-productive sequences. In contrast, 24 out of 26 $V_{\gamma 5}$ rearrangements were productive (Fig. 2c). The majority of these sequences contained from 1–8 NGE nucleotides and the ends of both the *V* and *J* segments were often shortened substantially, generating considerable diversity in the junctions. A codon for tyrosine is present in many of the $V_{\gamma 5}$ - $J_{\gamma 1}$ junctions (Fig. 2d), suggesting a possible shared specificity for an antigen or restriction element.

IEL δ -chain genes were amplified using *V*-region primers that correspond to seven published V_{δ} gene segments^{12,21,22}, in combination with the $J_{\delta 1}$ and $J_{\delta 2}$ primers. Large amounts of product were obtained with $V_{\delta 4}$ and $V_{\delta 6}$ primers and smaller or miniscule amounts with the others (not shown). This is consistent with the levels of messenger RNA observed on northern blot analysis (not shown), and indicates that the predominant V_{δ} gene segments used by IEL are a subset of the total V_{δ} pool²¹. It is apparent that all the various mechanisms for increasing TCR gene junctional diversity are fully used in IEL δ -chain rearrangements (Fig. 3), including use of multiple *D* and different *J* gene segments, insertion of NGE nucleotides, and imprecise joining^{1,18}. The extent of such diversity is very similar to that seen in the δ -chain rearrangements of adult thymocytes²¹ or splenic γ/δ T cells²³, again in sharp contrast to the limited diversity of dEC^{6,9} or early fetal thymocyte δ -chain rearrangements¹².

We have previously suggested that the development of T cells

within the thymus may be divided into two broad periods^{6,7,24}. In the first phase, the relative 'immaturity' of the recombinase machinery and a restricted pool of accessible gene segments may limit the extent to which TCR repertoires can be broadened^{12,13}. During the later phase, use of a larger subset of all germline gene segments, as well as the presence of the 'mature' recombination machinery combine to yield an extremely diverse population of TCRs^{18,21,23}. On the basis of the data reported here, we suggest that dEC represent the products of the earlier stage of T-cell development, and IEL are produced during the later, more mature phase.

The difference in the level of TCR diversity may provide insight into the immunological role of these two types of T cells. The normal functioning of the intestinal epithelium involves constant contact with a wide variety of biological compounds and microorganisms, and pathogenic invasion of this tissue may be comparatively frequent. Recent studies have indicated that IEL may be activated *in situ* to acquire cytolytic capability by exposure to normal intestinal biota¹⁷. The TCR repertoire of IEL may therefore need to be large in order to encompass this spectrum of bacteriological antigens. The normal function of the skin, however, is to serve as a barrier, isolating the epidermis and lower tissues from the environment. Contact of any one dEC with many different antigens may be rare, limiting the utility of a highly diverse TCR repertoire. Dendritic epidermal cells may instead recognize a common cellular product generated in response to pathogenic exposure. We have speculated that a possible target for this 'trauma signal surveillance' by dEC might be endogenous heat-shock proteins, induced in epidermal cells by a variety of cellular insults^{6,25}. It has recently been found that many γ/δ T cells react to mycobacterial heat-shock proteins^{26,27}, a possibility we are currently exploring for dEC. □

a			
	V	N	J
GERMLINE $V_{\gamma 2}$	TCCTACGGCTAAAG		
GERMLINE $J_{\gamma 1}$			ATAGCTCAGGTTTT
2.1.13	TCCTACGGCTA	CT	ATAGCTCAGGTTTT +
2.1.4	TCCTACGGCTA	GAGAT	ATAGCTCAGGTTTT -
2.2.1	TCCTACGG	TCT	ATAGCTCAGGTTTT -
2.2.5	TCCTACGGCTAAAG	AG	TAGCTCAGGTTTT -
2.2.6	TCC	CCCTAT	ATAGCTCAGGTTTT -
2.2.7	TCCTACGGCTA	GGAACAGTAT	ATAGCTCAGGTTTT -
2.2.8	TCCTACGGCTAAAG	CTAT	ATAGCTCAGGTTTT -
2.2.9	TCCTACGG	TCCT	ATAGCTCAGGTTTT -
2.2.11	TCCTACGGCTA	CGGTGGG	TAGCTCAGGTTTT -
2.2.12	TCCTACGG	TGAT	ATAGCTCAGGTTTT -
2.2.13	TCCT	CCGT	ATAGCTCAGGTTTT -
2.2.16	TCCTACGGCTAAA	C	TAGCTCAGGTTTT -

b			
	V	N	J
GERMLINE $V_{\gamma 4}$	TGTGCATGCTGGGATA		
GERMLINE $J_{\gamma 1}$			ATAGCTCAGGTTTT
4.1.3	TGTGCATGCTGGG	GAT	ATAGCTCAGGTTTT + (9)
4.1.2	TGTGCATGCTGGG	GATC	TAGCTCAGGTTTT +
4.1.8	TGTGCATGCTGGG		ATAGCTCAGGTTTT +
4.1.4	TGTGCATGCTGGGAT	C	TAGCTCAGGTTTT - (11)
4.1.1	TGTGCATGCTGGGAT	TT	ATAGCTCAGGTTTT - (3)
4.1.13	TGTGCATGCTGGG	GTAT	ATAGCTCAGGTTTT - (2)
4.1.6	TGTGCATGCTGGG	GTAC	ATAGCTCAGGTTTT -
4.1.24	TGTGCATGCTGGGA	ATT	ATAGCTCAGGTTTT -

c			
	V	N	J
GERMLINE $V_{\gamma 5}$	GCCTCCTGGGCTGCA		
GERMLINE $J_{\gamma 1}$			ATAGCTCAGGTTTT
5.1.1	GCCTCCTGGGCTGCA	T	ATAGCTCAGGTTTT + (5)
5.1.7	GCCTCCTGGG	GAT	ATAGCTCAGGTTTT + (3)
5.1.6	GCCTCCTGGGCT	T	ATAGCTCAGGTTTT + (2)
5.1.2	GCCTCCTGGG	TTGT	ATAGCTCAGGTTTT +
5.1.3	GCCTCCTGGG	GCTCAGGTTTT	+
5.1.5	GCCTCCTGGGCTGG	CGGG	ACCTCAGGTTTT +
5.1.8	GCCTCCTGGG	GGT	ATAGCTCAGGTTTT +
5.1.9	GCCTCCTGGGCT	CCGCTCG	GCTCAGGTTTT +
5.1.10	GCCTCCTGGGCTGG	CCG	TAGCTCAGGTTTT +
5.1.11	GCCTCCTGGG	TAT	ATAGCTCAGGTTTT +
5.1.12	GCCTCCTGGGCTGG	GAATCGAT	ATAGCTCAGGTTTT +
5.1.14	GCCTCCTGG	CAT	ATAGCTCAGGTTTT +
5.1.16	GCCTCCTGGGCTG	TAT	ATAGCTCAGGTTTT +
5.1.19	GCCTCCTGGG	GGGAG	GCTTTTT +
5.1.20	GCCT	TAT	ATAGCTCAGGTTTT +
5.1.22	GCCTCCTGGG	CCCAT	ATAGCTCAGGTTTT +
5.1.30	GCCTCCTGGGCTG	CT	ATAGCTCAGGTTTT +
5.1.13	GCCTCCTGGG	G	ATAGCTCAGGTTTT +
5.1.18	GCCTCCTGGGCTG	CCAAGGCC	ATAGCTCAGGTTTT -

d			
	V	N	J
GERMLINE $V_{\gamma 5}$	ASWAG		
GERMLINE $J_{\gamma 1}$			SSGF
5.1.1	ASWAG	Y	SSGF (5)
5.1.7	ASW	GY	SSGF (3)
5.1.6	ASWA	Y	SSGF (2)
5.1.2	ASW	LY	SSGF
5.1.3	ASW	G	SGF
5.1.5	ASWAG	G	SSGF
5.1.8	ASWA	GY	SSGF
5.1.9	ASWA	PSG	SGF
5.1.10	ASWAG	R	SSGF
5.1.11	ASW	VY	SSGF
5.1.12	ASWAG	NRY	SSGF
5.1.14	ASWA	AY	SSGF
5.1.16	ASWA	VY	SSGF
5.1.19	ASW	GE	GF
5.1.20	A	LY	SSGF
5.1.22	ASWA	PY	SSGF
5.1.30	ASWAG	Y	SSGF

FIG. 2 Junctional sequences of γ -chain rearrangements in IEL. a, $V_{\gamma 2}$ junctions. b, $V_{\gamma 4}$ junctions. c, $V_{\gamma 5}$ junctions. d, Peptide sequences encoded by IEL $V_{\gamma 5}$ junctions.

METHODS. Genomic DNA in crude cell lysates of IEL¹⁷ from C57Bl/6J mice was amplified by PCR with primers specific for $J_{\gamma 1}$ and either $V_{\gamma 1}$, $V_{\gamma 2}$, $V_{\gamma 3}$, $V_{\gamma 4}$ or $V_{\gamma 5}$. Products were obtained only with the $V_{\gamma 2}$, $V_{\gamma 4}$ and $V_{\gamma 4}$ and $V_{\gamma 5}$ primers. The $V_{\gamma 3}$ and $J_{\gamma 1}$ primers and subcloning and sequencing of the products, are as described in the legend to Fig. 1. Other primers were: ACATTGGTACCGGCAAAAAAC($V_{\gamma 1}$), GGGGGGAATTCCCCTACCCATATTTT-CTT($V_{\gamma 2}$), CCAAAGAATTCTGTAGTTC($V_{\gamma 4}$) and TCCACTGGTACCGATTCC-CAAGAA($V_{\gamma 5}$). The $V_{\gamma 1}$ primer hybridizes both to the $V_{\gamma 1.1}$ and $V_{\gamma 1.2}$ gene segments. The $J_{\gamma 1}$ primer hybridizes to all four known J_{γ} gene segments.

a	GERMLINE		N1	D δ 1	N2	D δ 2	N3	J δ 1	b	GERMLINE		N1	D δ 1	N2	D δ 2	N3	J δ 2
				GTGGCATATCA		ATCGGAGGATACGAG		CTACCGACAAA					GTGGCATATCA		ATCGGAGGATACGAG		CTCCTGGGAC
1.	V1	TGTGGGTC	CCCC	GCAT				CCGACAAA +	1.	V1	TGTGGGTCAGATA	C	GG		GGGA		CTCCTGGGAC +
2.	V1	TGTGGGTCAGA	CTTCT			ATCGGAGGATACGAG		CTACCGACAAA -	2.	V2	TGTGGAGGGAAA	CCCCCT	TC	CG	CGGAGGGATACGAG		CTCCTGGGAC +
3.	V1	TGTGGGTCAGAG	CCGATA	TGGCAT	CCCATCGCA	GGATACGAG	CCTC	CCGACAAA -	3.	V4	TGTGCTCTCATGGAGC	TCG			ATCGGAGGGATACG	CGAAG	CTCCTGGGAC +
4.	V1	TGTGGGTCAGAT	C	CATATC		GGAGGGATACG	GG	CTACCGACAAA -	4.	V4	TGTGCTCTCAT	CCC	TAT		ATCGGAGGGATACGAG	ATAC	CCTGGGAC +
5.	V1	TGTGGGTCAG		GG	AT	ATCGGAGGGATACG	TTT	CCGACAAA +	5.	V5	TGTGCTCTCGGGTAT	A			CGGAGGGATACGAG		CTCCTGGGAC -
6.	V2	TGTGGAGGGAAA		CCA	A	AGGG	GG	CTACCGACAAA +	6.	V6	TGTGCTCTCGGAACT	AA	AT		ATCGGAGGGATACGAG	TCAAG	CTCCTGGGAC -
7.	V2	TGTGGAGGGAAA	GA	GG	TAGG	CGGAGGG	CAACG	CTACCGACAAA -	7.	V7	TGTGCTCTCGGAACT	CT			ATCGGAGGGATACGAG		CTCCTGGGAC +
8.	V2	TGTGGAGGGAAA		TG	CT	GGGATACGAG	CT	CCGACAAA +	8.	V7	TGTGCTCTGAG		GG		ATCGGAGGGATACGAG		CTACCGACAAA -
9.	V2	TGTGGAGGAA	T			TGGAGGGATACG	GS	ACCGACAAA +	9.	V7	TGTGCTCTGAG	TG			ATA	GGGGATG	CCGACAAA -
10.	V3	TGTATCTGAGA	CT			ATCGGAGGG	GCCGC	CCGACAAA +	10.	V7	TGTGCTCTGAG						CTACCGACAAA +
11.	V3	TGTATCC	CTCTATA	TGGCATAT		ATCGGAGGGATACGAG	CTTC	ACCGACAAA +	11.	V7	TGTGCTCTGAG						CTACCGACAAA +
12.	V3	TGTATCTGAG	CTCCCAT	TGGC	GCCTGT	ATCGGAGGG	CCAA	TACCGACAAA +	12.	V7	TGTGCTCTGAG						CTACCGACAAA +
13.	V3	TGTATCTGAGAG	C			CGGAGGG	TCAA	CTACCGACAAA +	13.	V7	TGTGCTCTGAG						CTACCGACAAA +
14.	V3	TGTATCTGA		TAT		ATCGGAGGGATACGAG	G	CAAGACAAA +	14.	V7	TGTGCTCTGAG						CTACCGACAAA +
15.	V3	TGTATCTGAGAG		ATATC		GGAGGGA		CTACCGACAAA +	15.	V7	TGTGCTCTGAG						CTACCGACAAA +
16.	V4	TGTGCTCTCATGG				GGGGATACGA	T	CTACCGACAAA +	16.	V7	TGTGCTCTGAG						CTACCGACAAA +
17.	V4	TGTGCTCTCATGGAGCG	CGGA	TGGCATAT		ATCGGAGGGATACG	CTG	CTACCGACAAA +	17.	V7	TGTGCTCTGAG						CTACCGACAAA +
18.	V4	TGTGCTCTCATGGAG	AG	TGGC	GATGG	GGGA	G	CTACCGACAAA +	18.	V7	TGTGCTCTGAG						CTACCGACAAA +
19.	V4	TGTGCTCTCATGGAGCG	C	GG	GAG	GGAGGATACG	T	TACCGACAAA +	19.	V7	TGTGCTCTGAG						CTACCGACAAA +
20.	V4	TGTGCTCTC		TGG	GAGCTG	GGGATAC	AAG	CTACCGACAAA +	20.	V7	TGTGCTCTGAG						CTACCGACAAA +
21.	V4	TGTGCTCTCATGGAGCG	AA			ATCGGAGGG	CCT	CTACCGACAAA +	21.	V7	TGTGCTCTGAG						CTACCGACAAA +
22.	V4	TGTGCTCTCATGGAG		TG	T	ATCGGAGGGATACGAG	CTCC	CTACCGACAAA -	22.	V7	TGTGCTCTGAG						CTACCGACAAA +
23.	V4	TGTGCTCTCATGGAGCG				TCCGAGGGATAC	T	CGACAAA +	23.	V7	TGTGCTCTGAG						CTACCGACAAA +
24.	V4	TGTGCTCTCATGG				GAGGGATACGA	T	CTACCGACAAA +	24.	V7	TGTGCTCTGAG						CTACCGACAAA +
25.	V4	TGTGCTCTCA		GC	C	ATCGGAGGGATACG	G	CTACCGACAAA +	25.	V7	TGTGCTCTGAG						CTACCGACAAA +
26.	V4	TGTGCTCTCATGGAG				ATCGGAGGGATA	AGG	ACCGACAAA +	26.	V7	TGTGCTCTGAG						CTACCGACAAA +
27.	V4	TGTGCTCTCATGGAGCG	GA	AT		ATCGGAGGGATACG	G	AA +	27.	V7	TGTGCTCTGAG						CTACCGACAAA +
28.	V4	TGTGCTCTC	CGC	TAT		ATCGGAGGGATA	G	CTACCGACAAA +	28.	V7	TGTGCTCTGAG						CTACCGACAAA +
29.	V5	TGTGCTCTCGGG	ACT			ATCGGAGGGATACGAG	GTT	CTACCGACAAA -	29.	V7	TGTGCTCTGAG						CTACCGACAAA +
30.	V5	TGTGCTCTCGGG	GAAG	TGGCATAT	ATAGG	GGAGGATACG	TAG	CTACCGACAAA +	30.	V7	TGTGCTCTGAG						CTACCGACAAA +
31.	V6	TGTGCTCTCTGGAGCT		GG		ATCGGAGGGA	GC	CTACCGACAAA +	31.	V7	TGTGCTCTGAG						CTACCGACAAA +
32.	V6	TGTGCTCTCTGGAGC	CC	TAT		ATCGGAGGGATAC	CTG	CTACCGACAAA +	32.	V7	TGTGCTCTGAG						CTACCGACAAA +
33.	V6	TGTGCTCTCTGGAGCT			GGC	TCCGAG	TA	CTACCGACAAA +	33.	V7	TGTGCTCTGAG						CTACCGACAAA +
34.	V6	TGTGCTCTCTGGAGCT			GG	TCCGAGGGA		TACCGACAAA +	34.	V7	TGTGCTCTGAG						CTACCGACAAA +
35.	V6	TGTGCTCTCTGGAGCT			GGCA	CCCC	G	CTACCGACAAA +	35.	V7	TGTGCTCTGAG						CTACCGACAAA +
36.	V6	TGTGCTCTCTGGAGC	C			ATCGGAGGGATA	TGGTG	CTACCGACAAA +	36.	V7	TGTGCTCTGAG						CTACCGACAAA +
37.	V6	TGTGCTCTCTGGAGC	CCTT			ATCGGAGGGATACGAG	G	CTACCGACAAA +	37.	V7	TGTGCTCTGAG						CTACCGACAAA +
38.	V6	TGTGCTCTCTGGAGCT	GGCGTA	TGGC	CCAGG	ATCGGAGGGATACGA	A	ACCGACAAA +	38.	V7	TGTGCTCTGAG						CTACCGACAAA +
39.	V6	TGTGCTCTCTGGAGCT		GG	AG	ATCGGAGGG	GCGCGG	CCGACAAA -	39.	V7	TGTGCTCTGAG						CTACCGACAAA +
40.	V6	TGGCTCTCTCGGAAT	C			ATCGGAGGGATACGAG		CTACCGACAAA +	40.	V7	TGTGCTCTGAG						CTACCGACAAA +
41.	V6	TGGCTCTCTCGGA	CA	TGGCAT		TCCGAGGGATACG	TGG	CGACAAA +	41.	V7	TGTGCTCTGAG						CTACCGACAAA +
42.	V6	TGGCTCTCTCGG	CTAGG	GGC		GGAG		CTACCGACAAA +	42.	V7	TGTGCTCTGAG						CTACCGACAAA +
43.	V6	TGGCTCTCTCGGA	CCG	AT		ATCGGAGGGATACG	GGGCC	A +	43.	V7	TGTGCTCTGAG						CTACCGACAAA +
44.	V6	TGGCTCTCTCGGAAT	CT			GGAGGGATACGAG	C	ACCGACAAA +	44.	V7	TGTGCTCTGAG						CTACCGACAAA +
45.	V6	TGGCTCTCTCGGAAT	CT			TCCGAGGGATACGAG		CTACCGACAAA +	45.	V7	TGTGCTCTGAG						CTACCGACAAA +
46.	V7	TGTGCTCTGAG		GG		GGAGGGATACGAG		CTACCGACAAA -	46.	V7	TGTGCTCTGAG						CTACCGACAAA +
47.	V7	TGTGCTCTGAG		TG	ATCCCTTTT	ATCGGAGGGATA		CTACCGACAAA +	47.	V7	TGTGCTCTGAG						CTACCGACAAA +
48.	V7	TGTGCTCTGAG				AG		CTACCGACAAA +	48.	V7	TGTGCTCTGAG						CTACCGACAAA +
49.	V7	TGTGCTCTGAG		TG		ATA	GGGGATG	CCGACAAA -	49.	V7	TGTGCTCTGAG						CTACCGACAAA +
50.	V7	TGTGCTCTGAG	G			GGAGGGATACGAG		CTACCGACAAA +	50.	V7	TGTGCTCTGAG						CTACCGACAAA +

FIG. 3 Junctional sequences of δ -chain rearrangements in IEL. *a*, Rearrangements involving $J_{\delta 1}$. *b*, Rearrangements involving $J_{\delta 2}$. *c*, Peptide sequences encoded by IEL δ junctions. Nucleotides between V_{δ} and J_{δ} germline sequences were assigned to $D_{\delta 1}$ or $D_{\delta 2}$ if two or more consecutive bases were present in the D_{δ} sequences. This was largely an arbitrary choice, as eight of the sixteen possible dinucleotides are present in either $D_{\delta 1}$ or $D_{\delta 2}$. The $V_{\delta 6}$ sequences represent at least two different members of this subfamily. METHODS. Complementary DNA was prepared by reverse transcription of total cytoplasmic RNA (1 μ g) from IEL of C57Bq/6J mice with a C_{δ} -specific primer⁶. After dilution, the cDNA was amplified by PCR with primers specific

for $J_{\delta 1}$ and $J_{\delta 2}$ and one of seven V_{δ} primers in each reaction. The $V_{\delta 1}$, $J_{\delta 1}$, and $J_{\delta 2}$ primers, and the subcloning and sequencing of the products, are as described in the legend to Fig. 1. Other primers used were: GGGGGATCC-TGGCAGCCTCGTCTCCTTGG ($V_{\delta 2}$), TATTGGTACCGACAGGTTCCTCTTC ($V_{\delta 3}$), GTTCTGGTACCAACAGCAAGGAGGGCAGG ($V_{\delta 4}$), GGGGGTACCGACAGTGG-CAACCGGCACTG ($V_{\delta 5}$), GGGGGATCCATCAGCCTGTGATTTC ($V_{\delta 6}$) and AGCC-TCAGGGTACCAACCTGT ($V_{\delta 7}$). The $V_{\delta 6}$ primer hybridizes to all members of the $V_{\delta 6}$ subfamily described to date^{9,21}. The $V_{\delta 7}$ gene segment has been previously described as $V_{\delta 2.3}$ (ref. 22).

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Detection of refolding conformers of complement protein C9 during insertion into membranes

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HUMAN complement protein C9 is a hydrophilic serum glycoprotein responsible for efficient expression of the cytotoxic and cytolytic functions of complement. It assembles on the surface of a target cell together with C5, C6, C7 and C8 to form the membrane attack complex (MAC) and therefore has to change structure to become an integral membrane protein¹⁻⁷. As the protein assumes a stable structure in an aqueous environment, the question arises as to how it can enter the hydrophobic interior of a membrane. During MAC assembly C9 polymerizes into a circular structure, termed poly(C9) (ref. 8), which is responsible for the cylindrical electron microscopic appearance of the MAC. The suggestion has been made that C9 must at least partly unfold in order to enter a membrane⁷⁻¹¹ and also that polymerization of the molecule is intimately linked to insertion and cytotoxicity¹¹⁻¹³. The extent of unfolding and the mechanism of polymerization are not understood, nor is it known precisely which parts of the molecule participate in the proposed structural changes. We have been able to capture refolding C9 conformers during membrane insertion with the help of sequence-specific anti-peptide antibodies. Some of these antibodies inhibit C9-mediated haemolysis but not C9 polymerization, while others have the opposite effect. This suggests that the two processes are independent.

We have previously described^{9,14} evidence that C9 shares certain features with many other soluble proteins that insert spontaneously into membranes, such as colicins¹⁵, lytic peptides^{16,17}, and precursor proteins¹⁸⁻²⁰ that are synthesized in the cytoplasm but are destined for location in specific organelles. Although the experimental evidence for the generation of intermediates during the insertion of precursor proteins, colicins, and C9 into membranes is good, it is frequently circumstantial: for instance, resistance to proteolysis. It is desirable, therefore, to have probes that can recognize refolding conformers directly when a water-soluble protein changes to its membrane-associated structure. We reasoned that the fine specificity of antibodies for differences in protein structures could provide such tools. As it is highly unlikely, however, that one could prepare specific immunogens from native C9 to generate antibodies against conformers of unknown structure, we prepared antibodies against sequence-specific peptides.

Twelve peptides were selected that might represent sequence regions in human C9 which are either important for functional activity or for the structural changes associated with functional activity. A more detailed discussion of why these particular peptides were chosen will be presented elsewhere. Here we describe the results that we have obtained with polyclonal antibodies raised in rabbits against eight peptides. Figure 1a and b shows the location of the peptides within the amino acid sequence of human C9 and demonstrates that all of them recognize C9 on an immunoblot. When more specific sandwich enzyme-linked immunosorbent assays (ELISA) were performed, however, it became clear that only three antibodies, anti-(371-380), anti-(400-414) and anti-(509-517), recognized monomeric

native C9 when captured by a monoclonal antibody (mAb) (Fig. 1c). Lack of recognition by the other antibodies was not caused by steric hindrance or occlusion of the binding site by the capturing antibody because the results were identical when either mAb-42 or mAb-47 were used. The former recognizes an epitope that includes amino acids 412-426 and the latter an epitope containing amino acids 29-34 (ref. 11). None of the antibodies reacted with other terminal complement proteins (data not shown).

To test whether any of these antibodies would recognize C9 when part of the MAC, we used flow cytometry to measure their

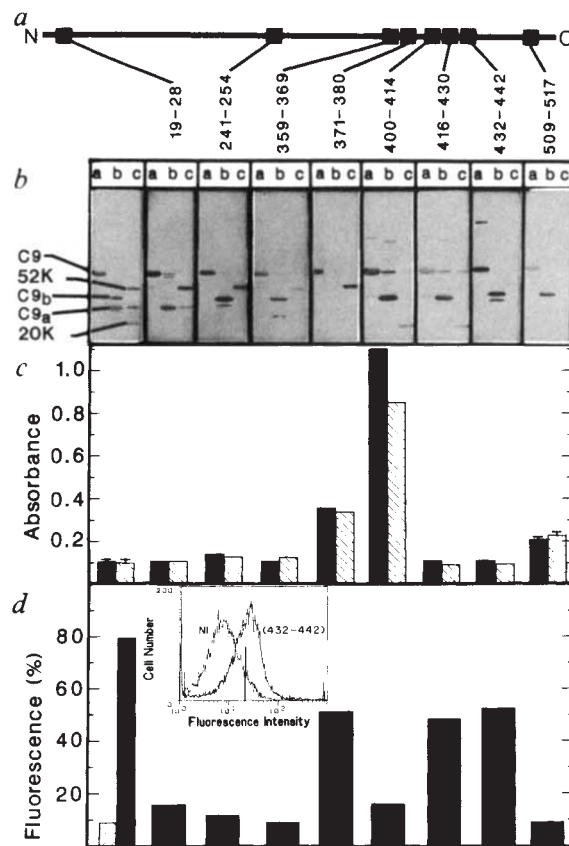


FIG. 1 Characterization of rabbit antibodies directed against C9-sequence specific peptides. a, Location of the selected peptides along the primary sequence of C9. b, Epitope mapping of the different antibodies by immunoblotting on whole C9 (lanes a) and fragments derived from limited proteolysis with α -thrombin (lanes b) or trypsin (lanes c). Far left panel shows a Coomassie blue staining of C9 and its fragments after transfer to a nitrocellulose membrane. c, Recognition of native C9 by anti-peptide antibodies in a 'sandwich' ELISA. Microtitre wells were coated with either monoclonal antibody mAb-42 (solid bars) or mAb-47 (hatched bars) to capture native C9 and binding of the anti-peptide antibodies was determined by ELISA. Far left panel shows binding of non-immune IgG to C9. d, Recognition of membrane-bound C9 by anti-peptide antibodies as measured by flow cytometry. A 'gate' was set at channel 20 and fluorescence recorded in channels 21-1000 is indicated for each anti-peptide. Far left panel shows results obtained with non-immune IgG (hatched bar) or polyclonal anti-C9 IgG (solid bars). Inset shows the autofluorescence of ghosts incubated with non-immune (NI) IgG contained in channels 1-20; specific binding of anti-peptide (432-442) shifts fluorescence to higher channels.

METHODS. Polyclonal rabbit anti-peptide antisera were elicited by immunization with peptides coupled to keyhole limpet haemocyanin, and monospecific IgG was isolated by immunoaffinity chromatography on solid phase peptides^{16,17}. C9 and proteolytic fragments were produced as described, and immunoblotting and sandwich ELISAs were performed using standard techniques^{16,17}. A Becton-Dickson FACS analyser was used for flow cytometry. Complement-lysed ghosts were prepared^{21,22}, mixed with individual anti-peptide antibodies, followed by addition of fluoresceinated IgG specific for rabbit IgG, and then analysed for ghost-associated fluorescence.

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binding to complement-lysed erythrocyte ghosts which carried the MAC. Again, three antibodies were found that bound to ghosts (Fig. 1d). Unlike anti-(371-380), however, which also recognizes native C9 as already described, the other two, anti-(416-430) and anti-(432-442), were incapable of recognizing native C9, whereas anti-(400-414) and anti-(509-517), which detect native C9, had no affinity for membrane-bound C9. When C9 is part of the MAC on a target cell it is thought to be polymerized into poly(C9) (refs 7, 21). Poly(C9) can be produced *in vitro* by incubation of C9 with ZnCl_2 ^{12,22}. When binding of anti-peptide antibodies to Zn-polymerized C9 was tested using a sandwich ELISA, only those antibodies that reacted with membrane-bound C9 also recognized poly(C9) (data not shown). We have therefore successfully produced antibodies which detect either native or membrane-bound C9.

The eight polyclonal anti-peptide antibodies were tested for their effect on C9-mediated lysis of antibody-sensitized erythrocytes carrying the earlier acting complement proteins C1-C8 (EAC1-8). Addition of C9 to a suspension of EAC1-8 cells elicits an immediate decrease in turbidity caused by haemolysis of the cells, and the time required to lyse 50% of the cells ($t_{50\%}$) can easily be monitored (Fig. 2a, inset). Despite the high affinity of C9 for EAC1-8 ($K_d < 10^{-11}$ M) (ref. 23), it is clear that anti-(416-430) and anti-(432-442) antibodies increased $t_{50\%}$ significantly, whereas anti-(371-380) and anti-(509-517) had a slight effect on haemolysis (Fig. 2a). The former two antibodies are those that recognized membrane-bound C9 and also poly(C9). Because of this specificity it was of interest to evaluate the effect they might have on the formation of poly(C9). Polymerization was triggered by incubation of monomeric C9 with a 17-fold molar excess of ZnCl_2 and the reaction was monitored by perpendicular-light scattering. In contrast to what was observed before, the two antibodies that inhibited haemolysis, anti-(416-430) and anti-(432-442), retarded aggregation only very slightly (Fig. 2b). Even more surprising, however, was the observation that two antibodies, anti-(19-28) and anti-(241-254), which had no detectable affinity for native or membrane-bound C9 or poly(C9) (see Fig. 1), inhibited polymerization very strongly. Poly(C9) formation was also suppressed by anti-(509-517), revealing that this reaction can be inhibited by antibodies directed against the amino and carboxy terminals and the middle section of the molecule.

When C9 aggregates it loses its haemolytic activity¹². We have verified this observation. When the different antibodies were evaluated for their influence on haemolytic activity of C9 in response to Zn-mediated polymerization, the results mirrored those obtained by monitoring aggregation directly (data not shown). In Table 1 we summarize the recognition patterns of all eight anti-peptide antibodies and their functional effects. We wish to emphasize the fact that we have produced two sets of antibodies, one comprised of anti-(19-28) and anti-(241-254) which inhibits poly(C9) formation but not haemolysis, and one comprised of anti-(416-430) and anti-(432-442) which inhibits

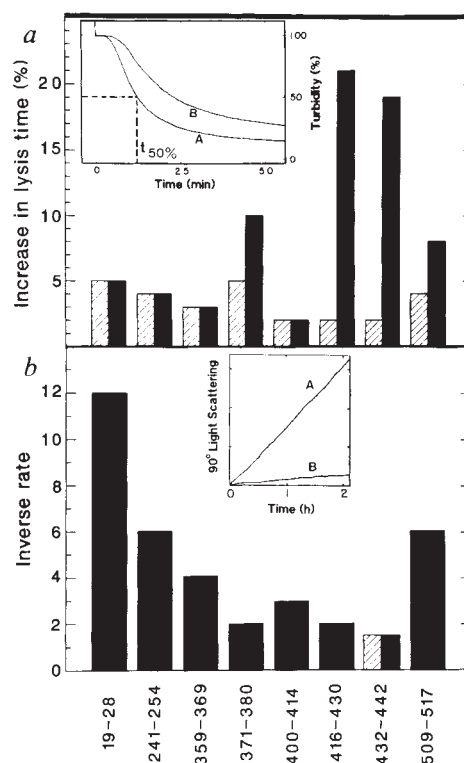


FIG. 2 Inhibition of C9-mediated lysis of EAC1-8 cells and of Zn^{2+} -catalysed polymerization of C9. a, Inset shows decrease in turbidity (curve A) caused by lysis of EAC1-8 cells effected by addition of C9 at time 0. Time required to achieve 50% lysis is indicated as $t_{50\%}$ and inhibition of lysis by curve B. The increase in $t_{50\%}$ values effected by anti-peptide IgG is displayed as solid bars and by pre-immune IgG as hatched bars. b, Inset shows increase in 90°-light scattering caused by formation of C9 polymers during incubation of C9 with Zn^{2+} (curve A); inclusion of anti-peptide (19-28) prevents polymerization of C9 (curve B). Inhibition of polymerization by the different antibodies (solid bars) or by non-immune IgG (hatched bar) is plotted as the inverse rate of polymerization for each antibody.

METHODS. EAC1-8 cells were prepared as described^{16,22} and lysis mediated by C9 was monitored by forward light scattering¹⁶. Polymerization of C9 (6 μM) catalysed by ZnCl_2 (0.1 mM) was performed as described¹² and the reaction was monitored by 90°-light scattering at 320 nm in a fluorometer. The inverse reaction rate was calculated from the initial slope between 0 and 30 min.

haemolysis but not polymerization, strongly suggesting that the two reactions are mutually independent.

To further evaluate the C9 conformer that could act as an antigen for anti-(19-28), we made use of the antibody-binding capacity of protein A coupled to Sepharose beads. These beads were first used to capture the different anti-peptide antibodies and then, after washing and resuspension, monomeric C9 (con-

TABLE 1 Recognition patterns of anti-peptide antibodies and their functional effects

Anti-peptide	Denatured C9*	Native C9†	MAC‡	Inhibits C9-mediated lysis	Inhibits C9 polymerization§
(19-28)	+	—	—	—	++
(241-254)	+	—	—	—	+
(359-369)	+	—	—	—	—
(371-380)	+	+	+	+	—
(400-414)	+	+	—	—	—
(416-430)	+	—	+	++	—
(432-442)	+	—	+	++	—
(509-517)	+	+	—	+	+

+, Inhibition; ++, strong inhibition.

Determined by: *, immunoblots; †, 'sandwich' ELISA; ‡, flow cytometry analysis of complement-lysed ghosts; §, 90°-light scattering.

taining ^{125}I -labelled C9 as a tracer) was added and the mixture incubated with or without a 17-fold molar excess of ZnCl_2 . As anti-(19-28) did not react with monomeric or poly(C9), we reasoned that a different C9 conformer must have formed during polymerization which was able to bind to the solid-phase antibody. The results shown in Fig. 3a verified our expectation: the amount of radioactive C9 captured by the beads increased significantly only when ZnCl_2 was present in the mixture. Analysis of the bound ^{125}I -labelled C9 by SDS-PAGE and autoradiography confirmed that it was not present as SDS-resistant poly(C9) (data not shown). In contrast to anti-(19-28), another antibody, anti-(509-517), which also inhibited poly(C9) formation, did not capture more radioactive C9. We conclude from these results that the latter antibody has a small inhibitory effect because it binds already to native monomeric C9 (see also Fig. 1c) and thereby inhibits aggregation, whereas anti-(19-28) suppresses aggregation because it traps a transient C9 conformer.

When the same experimental protocol was used with anti-(432-442) bound to protein A-Sepharose, it also precipitated significantly more ^{125}I -labelled C9 when polymerization was allowed. This result was not expected because the antibody does not have any influence on Zn-catalysed C9 aggregation (Fig. 2b). Because the antibody retarded haemolysis, however, (Fig. 2a) the combined data would be consistent with the notion that during poly(C9) formation and during haemolysis, the same C9 conformer was formed. To test this possibility, we modified the Protein-A-Sepharose experiment in such a way that recognition of the same conformer by different antibodies could be tested. Solid phase anti-(19-28) was used to trap the (unlabelled) C9 conformer and then binding of a second radioiodinated anti-peptide IgG to the trapped conformer was measured. Only anti-(371-380), which recognizes all forms of C9 (Table 1), associated with the trapped conformer, whereas anti-(416-430) and anti-(432-442) did not (Fig. 3b). As we will report elsewhere, some of the antibodies that do not recognize native or membrane-bound C9 also accumulate during lysis on target cells. These results indicate that several different conformers are formed during C9 aggregation *in vitro* and on membranes during haemolysis. The existence of such transient intermediates was

originally predicted by Boyle and Borsos³, who described them as C9^{'doomed'} and C9^{'inserted'}. We have now been able to provide direct experimental proof for these predictions. Our results do not support models which link haemolysis directly to C9 polymerization.

In summary, we have found several anti-peptide antibodies which do not recognize soluble native C9 or the final membrane-bound form. They do, however, capture C9 conformers that are formed during membrane insertion of C9, as well as conformers formed during *in vitro* polymerization. This shows that anti-peptide antibodies specific for refolding conformers can be generated and that such antibodies are very useful tools to unravel some of the intricacies of protein reactions on membranes. This approach may also be useful for study of protein folding and to provide information on protein conformers in general. □

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Augmentation of cardiac calcium current by flash photolysis of intracellular caged- Ca^{2+} molecules

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THE entry of calcium ions into cells through voltage-activated Ca^{2+} channels in the plasma membrane triggers many important cellular processes. The activity of these channels is regulated by several hormones and neurotransmitters, as well as intracellular messengers such as Ca^{2+} itself (for examples, see refs 1-9). In cardiac muscle, myoplasmic Ca^{2+} has been proposed to potentiate Ca^{2+} influx^{1,7-9}, although a direct effect of Ca^{2+} on these channels has not yet been demonstrated. Photosensitive 'caged- Ca^{2+} ' molecules such as nitr-5, however, provide powerful tools for investigating possible regulatory roles of Ca^{2+} on the functioning of Ca^{2+} channels^{10,11}. Because its affinity for Ca^{2+} is reduced by irradiation, nitr-5 can be loaded into cells and induced to release Ca^{2+} with a flash of light¹⁰. By using this technique we found that the elevation of intracellular Ca^{2+} concentration directly augmented Ca^{2+} -channel currents in isolated cardiac muscle cells

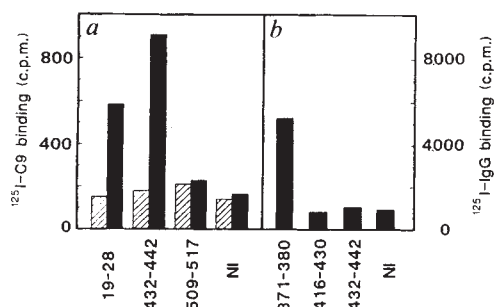


FIG. 3 Detection of C9 conformers. *a*, Binding of C9 to solid-phase anti-peptide IgG during Zn^{2+} -catalysed C9 polymerization. Solid phase anti-peptide IgG or non-immune (NI) IgG was first prepared by fixing antibody (1.5 mg) to a suspension of protein A-Sepharose 6MB beads (Sigma) in 70 μL buffer (10 mM Tris, 0.15 M NaCl, pH 7.3). After washing away unbound antibody, the beads were resuspended in 0.9 ml. Tris buffer containing 0.1 mM ZnCl_2 . Monomeric, ^{125}I -trace-labelled C9 (40 μg) was added and the mixture incubated for one hour with mixing. The beads were washed and the associated radioactivity was measured (solid bars). Control samples (hatched bars) were treated identically, except ZnCl_2 was omitted during the incubation. *b*, Binding of two anti-peptide antibodies to a trapped C9 conformer captured by anti-(19-28) during Zn^{2+} -catalysed C9 polymerization. Anti-peptide (19-28) was bound to Protein A-Sepharose beads and incubated with unlabelled C9 in a ZnCl_2 containing Tris buffer as described before. After removing unbound antibody, ^{125}I -trace-labelled anti-peptide IgG or non-immune IgG was added and after one hour, bead-associated radioactivity was determined. All measurements shown in this figure are the means of triplicate determination.

from both frog and guinea pig. The time course of the current potentiation was similar to that seen with β -adrenergic stimulation. Thus Ca^{2+} may work through a similar pathway, involving phosphorylation of a regulatory Ca^{2+} -channel protein. This mechanism is probably important for the accumulation of Ca^{2+} and the amplification of the contractile response in cardiac muscle, and may have a role in other excitable cells.

Although a few experiments have been attempted with isolated ventricular cells from guinea-pig heart, most have been with frog atrial cells, from *Rana temporaria* or *Rana esculenta*, because the sarcoplasmic reticulum in these cells is less well developed¹². The whole-cell tight-seal recording technique was used to voltage clamp cells, and nitr-5 (2 mM) was incorporated intracellularly by its inclusion in the solution filling the patch pipettes¹⁰. In most experiments, nitr-5 was the only Ca^{2+} chelator present, and CaCl_2 was added to set the pre-flash internal free- Ca^{2+} concentration at ~ 145 nM. Light flashes, delivered from a xenon short-arc flashlamp, were focused onto cells through the epifluorescence port and objective of an inverted microscope. A single flash was estimated¹⁰ to raise the concentration of free Ca^{2+} to ~ 1 μM . Because the light spot was restricted to the cell and <200 μm of the pipette tip, unphotolysed solution from the pipette would have rapidly returned the Ca^{2+} concentration to the pre-flash level, probably within about 30 s (ref. 10).

A single light flash resulted in strong enhancement of the current carried through Ca^{2+} channels by either Ca^{2+} or Ba^{2+} (Fig. 1a, b). In frog atrial cells, the flash increased the Ba^{2+} current amplitude by a factor of 2.6 ± 0.3 (mean $\pm$ s.e.m.; $n = 5$), whereas Ca^{2+} currents were increased by a factor of 2.0 ± 0.2 ($n = 3$). The Ca^{2+} current was similarly potentiated in guinea-pig ventricular cells (Fig. 1c), with a mean increase in peak amplitude of 1.6-fold ($n = 2$). This effect was reproducible and fully reversible, with the current returning to the pre-flash level in 5–10 min (results not shown). Current potentiation reflected the rise in intracellular Ca^{2+} because flashes had no effect (1) when nitr-5 was replaced with EGTA (1–10 mM; results not shown), or (2) when CaCl_2 was omitted and an excess of the photostable chelator BAPTA (10 mM), which binds Ca^{2+} rapidly and tightly¹¹, was added to the pipette (Fig. 1c). Changes in pH were unlikely to have been responsible for the potentiation seen with nitr-5, because nitr-5 has a low affinity for protons at physiological pH^{11,13}, and therefore should not have bound to protons when Ca^{2+} was released. If, on the contrary, raising the Ca^{2+} concentration caused a fall in intracellular pH, as previously shown in the mammalian heart¹⁴, then this would have been expected to block Ca^{2+} channels¹⁵. Furthermore, changes in pH should have been quickly buffered by HEPES (10 mM) included in the pipette. Finally, flash photolysis of intracellular caged cyclic GMP (≈ 250 μM), which releases protons as a by-product, has been previously shown to have no effect on Ca^{2+} currents in the frog atrium¹⁶. Thus we conclude that the potentiation of the Ca^{2+} current by flash photolysis of caged Ca^{2+} arose as a result of released Ca^{2+} .

Figure 2a compares the current-voltage relationship of peak Ca^{2+} -channel currents, recorded from a frog atrial cell before and after nitr-5 photolysis. Frog atrial¹⁷ and guinea-pig ventricular¹⁸ cells display two components of Ca^{2+} current: from holding potentials of -80 to -100 mV, depolarizing steps of increasing amplitude activate first a low-threshold component, which peaks at around -40 mV, and then a high-threshold component that begins to activate at around this voltage. In a cell with a negligible low-threshold current (Fig. 2a), the peak current amplitude was enhanced by Ca^{2+} release induced by a flash at all potentials. At potentials too negative to activate high-threshold current, however, the flash had no measurable effect. This implies that the increased current was carried through high-threshold Ca^{2+} channels. In agreement with this, both the pre-flash and flash-induced currents were blocked by low (<100 μM) concentrations of Cd^{2+} (Fig. 2b). Thus the increase

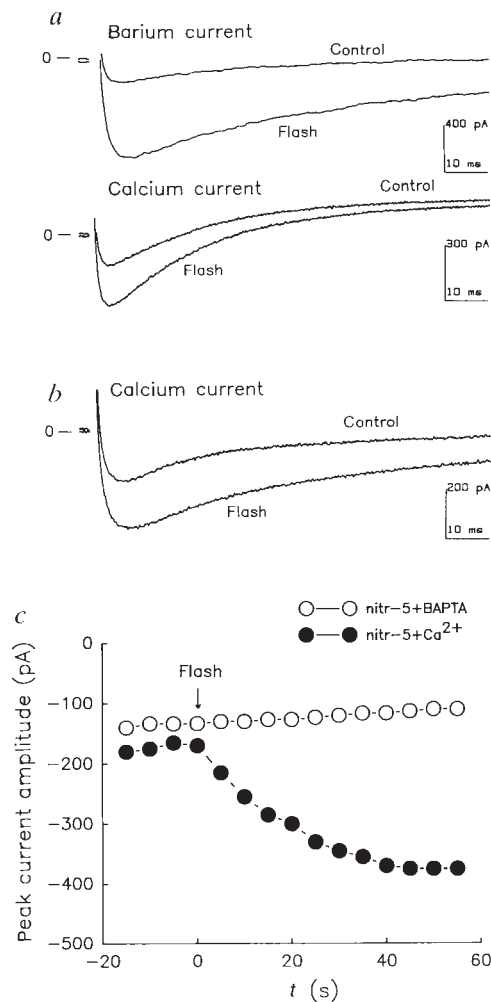


FIG. 1 Flash photolysis of caged Ca^{2+} augments Ca^{2+} -channel currents in frog atrial (a) and guinea-pig ventricular (b) cells. Currents evoked by voltage steps to $+10$ mV from a holding potential of -80 mV (frog) or -50 mV (guinea pig) before a flash (control) and at the peak of the response. Blanked periods indicate amplifier saturation from capacity transients. Leak currents are not subtracted. c, Effect of buffering intracellular Ca^{2+} . Data from two frog atrial cells stimulated at 0.2 Hz. Pipettes contained either 2 mM nitr-5 with added Ca^{2+} (1 mM) (●), or 2 mM nitr-5 with 10 mM BAPTA (○). Current amplitude is measured as difference between peak current and steady-state current at end of 150-ms pulse.

METHODS. Frog atrial cells were isolated by a similar method to that described in ref. 28. In brief, tissue fragments were incubated for 15 min in a solution containing (mM): NaCl, 109; KCl, 2.5; MgCl_2 , 7.5; CaCl_2 , 0.15; NaHCO_3 , 2; glucose, 10; HEPES, 10; pH 7.4. They were then incubated for 1 h in the same solution but without NaHCO_3 and containing pyruvate (10 mM), taurine (10 mM), trypsin (500 international unit (IU) ml^{-1} ; type III, Sigma), collagenase (200 IU ml^{-1} ; type 1A, Sigma), protease (2 IU ml^{-1} ; type XIV, Sigma) and bovine serum albumin (BSA; 0.05% fraction V, Sigma). Finally, fragments were incubated for at least 3 h in the first solution with 0.05% BSA. Guinea-pig ventricular cells were isolated as described²⁹. For recording, frog cells were bathed in (mM): NaCl, 109; KCl, 2.5; MgCl_2 , 2.5; CaCl_2 (or BaCl_2), 5; NaHCO_3 , 2; glucose, 10; HEPES, 10; tetraethylammonium, 5; tetrodotoxin, 0.001; pH 7.4. Pipettes (2–6 M Ω) usually contained (mM): CsCl, 110; nitr-5, 2; CaCl_2 , 1; ATP-Mg, 1; GTP-Na, 0.5; phosphocreatine, 5; pH 7.2. All but 1–6 M Ω of series resistance was compensated. Similar solutions were used for guinea-pig cells, except that extracellular NaCl and intracellular CsCl were increased to 130 mM and extracellular CaCl_2 was reduced to 1 mM. The flashlamp (Chadwick Helmut, El Monte, California) discharged 230 J of stored energy in <1 ms over a broad spectrum, which was filtered to remove wavelengths <300 nm before focusing. Changes in Ca^{2+} concentration were estimated from flash-induced changes in absorbance of test droplets (~ 250 μm diameter) of nitr-5 solution placed in the set-up as previously described¹⁰. All experiments performed at room temperature (~ 22 – 24 $^{\circ}\text{C}$).

in current induced by a flash must have been caused by an increase in Ca^{2+} -channel activity.

The flash-induced current developed slowly, taking many seconds to reach the maximal effect (see Figs 1c and 2b). This did not, however, reflect the speed of Ca^{2+} release from nitr-5, because this occurs with a sub-millisecond time constant¹¹. It was more likely to reflect intracellular events coupling the rise in Ca^{2+} concentration to current potentiation. Further experiments were thus performed on frog atrial cells to determine the possible nature of these intracellular events. The slow increases in the Ca^{2+} -channel current were reminiscent of responses to β -adrenergic agents such as isoprenaline, which act primarily through cyclic AMP-dependent phosphorylation of regulatory channel proteins¹⁹. Perhaps channels could also be phosphorylated by Ca^{2+} -dependent protein kinases, which are known to be present in heart muscle²⁰. Thus, it was of interest to compare directly the time course of current potentiation induced by a flash with that induced by isoprenaline. Figure 3a shows such a comparison made in a single cell; an application of isoprenaline ($10\text{ }\mu\text{M}$ for 10 ms) increased the Ca^{2+} current by a factor of 5.1 ± 0.8 (mean $\pm$ s.e.m; $n = 10$). The increase in response to isoprenaline occurred with a delay that was not apparent in the response to the flash. Ignoring this, however, isoprenaline produced an increase in the current over a similar time course to flash-induced Ca^{2+} release. The delay presumably reflected the diffusion time of isoprenaline from the pipette to the cell and the activation of adenylate cyclase, because it can be removed if the adrenergic receptors are by-passed by applying cAMP directly in cells, using flash-photolysis of caged cAMP^{16,21}. The responses to a flash and to isoprenaline also appeared to follow a similar time course in guinea-pig ventricular cells.

If the transient release of Ca^{2+} causes phosphorylation of the same protein as does isoprenaline, then channels maximally activated by isoprenaline should not respond to a flash²². Figure 3b shows that when a flash was presented at the peak of the response to isoprenaline, it did not augment the current any further. A similar result was obtained when the stimuli were applied in the reverse order, with a flash reducing the effectiveness of isoprenaline (results not shown). Thus there seems to be a common step in the pathways leading to the Ca^{2+} -current potentiation by flash-induced Ca^{2+} release and isoprenaline. If Ca^{2+} in some way triggers channel phosphorylation, then the response to a flash should depend on the intracellular level of ATP, as shown for isoprenaline²². Indeed, if ATP-Mg was omitted from the pipette, the flash-induced Ca^{2+} release only increased currents by $\sim 30\%$ in frog atrial cells. The responses also showed 'rundown', in that flashes were less effective on the second and subsequent presentations. In the presence of ATP, however, repeated flashing (or isoprenaline application) at 5–10 min intervals produced almost identical increases in current.

Does this phenomenon have a physiological role? It could underlie the facilitation of the Ca^{2+} current observed in mammalian^{7,9,23,24} and frog⁸ heart cells during repetitive stimulation. This is associated in frog atria with an increase in contractile force^{8,25}. Stimulation-dependent current potentiation requires Ca^{2+} influx, because it is suppressed when other ions carry the current^{7,8}, and it occurs over a similar time course to the potentiation described here. This facilitation is also blocked by isoprenaline⁷. A similar Ca^{2+} -dependent facilitation mediates the enhancement of the cardiac Ca^{2+} current produced by low concentrations of cardiac glycosides¹, and may have additional roles in maintaining Ca^{2+} -channel activity⁹. Thus positive regulation of the Ca^{2+} current by intracellular Ca^{2+} could provide a general mechanism for accumulating Ca^{2+} and amplifying the contractile response in cardiac muscle.

To prevent intracellular Ca^{2+} overloading, sufficient increases in cytoplasmic Ca^{2+} concentration might be expected to eventually inactivate Ca^{2+} channels^{2–6}. In many previous experiments, inactivation may have been large enough to mask the facilitatory effects of Ca^{2+} (ref. 7). Nevertheless, the predominant effect of

flash-induced Ca^{2+} release was to potentiate the Ca^{2+} -channel current. In frog cells, inactivation was usually seen only when currents were already potentiated by isoprenaline (Fig. 3b). By contrast, the flash-induced potentiation in guinea-pig cells was preceded by a small ($<10\%$) and short-lived ($<10\text{ s}$) depression of the Ca^{2+} -current amplitude. These findings are consistent with the original proposal of Marban and Tsien¹ that relatively small rises in myoplasmic Ca^{2+} concentration facilitate Ca^{2+} influx, whereas larger rises oppose it. Unfortunately, the present experiments do not elucidate the absolute concentrations at which Ca^{2+} produces the two effects. Our estimate of $1\text{ }\mu\text{M}$ as the concentration of Ca^{2+} generated by a flash may not reflect the true change detected by the cells. The transient release of Ca^{2+} may have been reduced if cell absorbance was significant and/or Ca^{2+} was rapidly bound by contractile proteins. The transient increase in Ca^{2+} would also be distorted by the sarcoplasmic reticulum, which releases Ca^{2+} in response to a rise in Ca^{2+} concentration²⁶, and is a particular problem when studying guinea pig cells. A quantitative description of Ca^{2+} regulation of Ca^{2+} channels will therefore require the intracellular concentration of free Ca^{2+} to be measured at the same time as it is changed.

The present study indicates that sarcoplasmic Ca^{2+} augments Ca^{2+} -channel currents by activating a protein kinase. The most

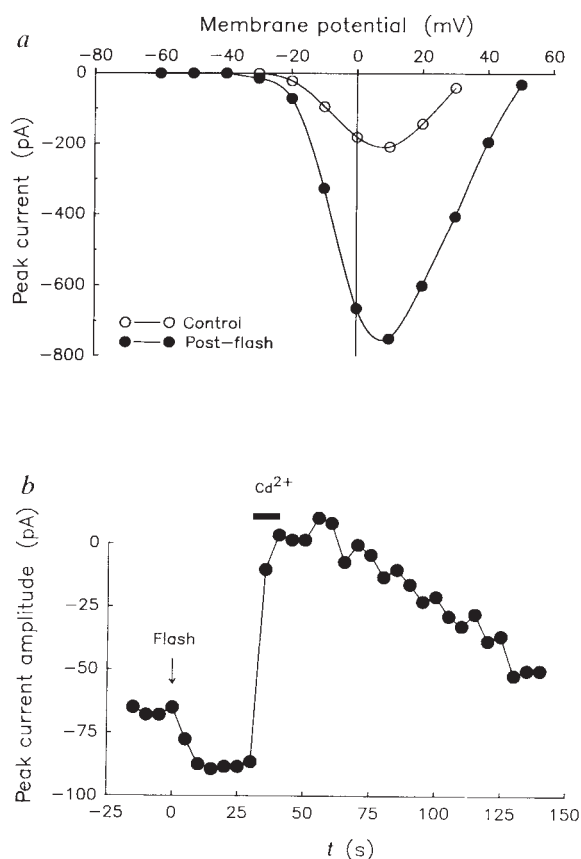


FIG. 2 Voltage and Ca^{2+} dependence of the flash-induced current in frog atrial cells. *a*, Voltage dependence of peak Ba^{2+} current during 200-ms voltage steps, from a holding potential of -80 mV to various test potentials. Currents recorded before the flash ($\circ$) and at the peak of the flash response ($\bullet$). *b*, Peak amplitude of Ca^{2+} current evoked by steps to $+10\text{ mV}$ from a holding potential of -80 mV at 0.2 Hz . After a flash (indicated by an arrow), delivered at $t = 0$, peak current amplitude increased. Bathing solution containing $100\text{ }\mu\text{M}$ CdCl_2 (application indicated by a bar) was applied to the cell during the flash response by pressure ejection ($7\text{--}20\text{ kPa}$) from a micro-pipette positioned $\sim 100\text{ }\mu\text{m}$ away. This blocked both the pre-flash and flash-induced current.

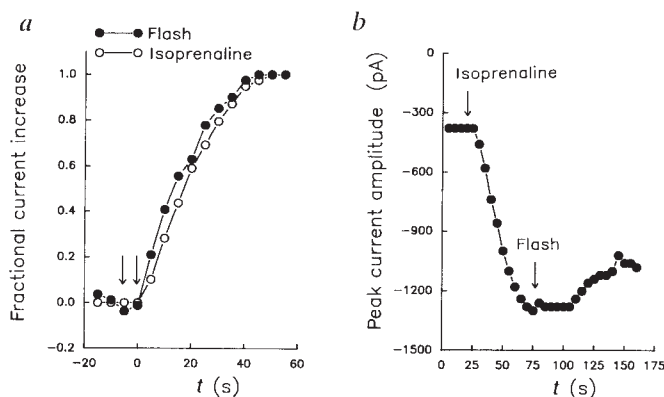


FIG. 3 Comparison of the increases in current induced by a flash and by 10 ms exposure to isoprenaline (10 μ M) in frog atrial cells. Currents elicited by depolarizing steps to +10 mV from a -80 mV holding potential at 0.2 Hz. Isoprenaline application is indicated by the arrow furthest to the left and flashes by the arrow furthest to the right. *a*, Time course of potentiation. The current was first increased by presenting a flash to the cell (●), allowed to recover, and then stimulated again by applying isoprenaline (○). The increase in peak current amplitude at each voltage step is plotted as a fraction of the maximum increase observed in response to either stimuli. The current recorded immediately before the first detectable change in amplitude is plotted as the value at $t=0$. *b*, Blockade of the flash-induced current potentiation by isoprenaline. Peak current amplitude was first increased by applying isoprenaline. A flash was then presented at the peak of the isoprenaline response. No further increase was observed.

likely candidate is a Ca^{2+} - and calmodulin-dependent protein kinase¹. This could promote phosphorylation by interacting with the cAMP cascade. Alternatively, the kinase could directly phosphorylate the channel. Support for this idea comes from a recent report that shows that dihydropyridine-sensitive Ca^{2+} channels in cell-free membrane patches from GH3 cells are modulated by Ca^{2+} -calmodulin-dependent phosphorylation²⁷. The approach that we used to study Ca^{2+} regulation of Ca^{2+} channels is one that can be employed to directly investigate these possibilities. □

Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases

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ALPHA atrial natriuretic peptide (α -ANP) and brain natriuretic peptide are homologous polypeptide hormones involved in the regulation of fluid and electrolyte homeostasis^{1,2}. These two natriuretic peptides apparently share common receptors and stimulate the intracellular production of cyclic GMP as a second messenger¹. Molecular cloning has defined two types of natriuretic peptide receptors: the ANP-C receptor of relative molecular mass (M_r) 60–70,000 (60–70 K), which is not coupled to cGMP production and may function in the clearance of ANP (refs 3, 4) and the ANP-A receptor of M_r 120–140 K, which is a membrane form of guanylate cyclase in which ligand binding to the extracellular domain activates the cytoplasmic domain of the enzyme^{5,6}. Here we report the cloning and expression of a second human natriuretic peptide-receptor guanylate cyclase, the ANP-B receptor. The ANP-B receptor is preferentially activated by porcine brain natriuretic peptide rather than human α -ANP, whereas the ANP-A receptor responds similarly to both natriuretic peptides. These observations may have important implications for our understanding of the central and peripheral control of cardiovascular homeostasis.

In the course of screening a human placental complementary DNA library for ANP-A receptor clones⁵, we identified a cDNA encoding a protein homologous to the ANP-A receptor, which we have termed the ANP-B receptor. The nucleotide- and predicted amino-acid sequence of the ANP-B receptor is shown in Fig. 1. This sequence probably represents a nearly complete transcript as northern analysis indicates that human brain ANP-B receptor messenger RNA is ~4,000 nucleotides long (data not shown). In the 283 nucleotides 5' of the putative translation initiation codon there are termination codons in all three reading frames. The translation initiation codon defines the beginning of a 1,048 amino acid open reading frame with an N-terminal hydrophobic signal peptide⁷ of 22 amino acids. Beginning with residue Arg+1, the mature ANP-B receptor amino-acid sequence has a predicted M_r of 114,952 with a 442-amino-acid extracellular domain containing 7 potential N-linked glycosylation sites and 6 cysteine residues. Hydrophobic analysis predicts a transmembrane domain from residues 434 to 456 followed by an Arg-Lys stop transfer sequence⁸. The predicted cytoplasmic domain extends for 569 amino acids and has one potential N-linked glycosylation site and 9 cysteine residues.

The predicted amino-acid sequences of the human ANP-B receptor, the human ANP-A receptor⁵ and the bovine ANP-C receptor³ are compared in Fig. 2. The extracellular domain of the ANP-B receptor has 44% amino-acid identity with the ANP-A receptor and 30% identity with the ANP-C receptor, whereas the ANP-A receptor has 33% identity with the ANP-C receptor^{5,6}. There is one region that is more highly conserved in all three molecules: residues 75–102 of the ANP-B receptor have 79% sequence identity with the ANP-A receptor and 71% identity with the ANP-C receptor. It is possible that the greater similarity found in this region may be critical in the recognition of natriuretic peptides. This region also contains one of the two cysteine residues that are conserved between all three receptors, ANP-B receptor residues Cys 79 and Cys 205. The structural and functional homology (see below) of the ANP-B, ANP-A,

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To examine the biochemical properties of the ANP-B receptor, we constructed an expression vector in which the coding sequence was placed under the transcriptional control of the

ANP-B 45 V . . . S S E . L E G A C S E Y L A P L S A V D L K L Y H D P D L L L G P G C V Y P A A S V A R F A
 ANP-A 46 T V L G S S E N A L G V C S D A P L A V D L K W E H N P A V F L G P G C V Y A A A P V G R F T
 ANP-C 50 Q . . . A Y E D S D C G N R A L F S L V D R V A A A R G A K P D L I L G P V C E Y A A A P V A R I A L

ANP-B 141 T A R A A L L Y L D A R T D D R P H Y F T I E G V F E A L Q G S - N L S V G H Q V Y A R E P G G P -
ANP-A 147 E R Q A L M V Y A R P G D E E N C F F L V E G L F M R V R D L N I T V D H L F E A E D D L S Y
ANP-C 153 S R - A V L V Y S D K - L E R N C F F T L E G V H E V F Q E - G L H T S A Y N F L K D L D L D L

ANP-B 229 S L R A G P T R A T G R P W Q D N R T R E G A A L R E A F Q T V L V I T Y R E P P N P E Y Q E F Q
ANP-A 261 S L Q G G G A P A R P R W E R G D G Q D V S A R G A Q A A K I T Y L K D P N P E Y L E F L
ANP-C 262 S F Y G D G S E W K R G D K H D F E A K I O A Y S L Q T I T L R T V K P E F E K I S

ANP-B 339 GLRIIVEKMOGRIRYHGVITGLVIMDKNNDRETDFVLWAMGDLDSGDIFQPAAH
ANP-A 345 QENITQRMIRNNSFFQGVITGLYIKDSSSGDRETDFSLWDMOPENGAERFVLN
ANP-C 346 GGGKLTQTNWRTEFEGGLACGVSKDANGDPRYDFSLVAMTDTFAGTQFVFLVD

ANK-B 437 V A L G T G I T F I M F G V S S F L I F R K L M L E K E L A S M L W R I R W E E L Q F G N S E R Y H
ANK-A 440 L A L V G S S L G L G I V S F F I Y R K M O L E K E L A S E L W R V R W E D V P S S L F R H L
ANK-C 443 L T V V G A L G A C I L M A E Y E F R K Y R V T I E R A S N O O F E S N U C K H O F I P D S I

ANT-B 537 ELTRQVLVFELKHMROVQFNHLTRFVGACIDPPNICILVEYCPRGSQDIL
ANT-A 543 ELTRKVLVFELKHMROVQNEHLTRFVGACTDPPNICILVEYCPRGSQDIL

ANP-B 637 K I T D Y G L A S F R S T A E P D D S H A L Y A K K L W T A P E L L S G N P L P T T G M Q K A D V Y
ANP-A 643 K I T D Y G L E S F R D L D P E Q G H T V Y A K K L W T A P E L L R M A S P P V R I G S Q A G D V Y

ANP-B 737 E E L V L L M E R C W A Q D P A E R P D F G I K G F I R R F N K E G G T S I L D N L L L M R M E Q Y
ANP-A 742 E E L G L L M Q R C W A E D P Q E R P P F Q I R L T L R K F N R E N S S N I L D N L L S R M E Q Y

ANP-B 767 A N N L E K L V E E A R T Q A Y L E E K R K A E A L L Y Q I L P H S V A E Q L K R G E T V Q A E A F D

ANP-B 807 GDAYMVVSGLPGRNGQRHAF E IARMALALLDAYSSFR I RHRPHDQLRLRI
ANP-A 892 GDAYMVVSGLPVRNGRLHACEVARMALALLDAYRSFR I RHRPQEQLRLRI

ANP-B3 987 L D E L G C F D I E L R G D V E M K G K G K M R T Y W L L G E R K G P P G L L
ANP-A 992 L E F E F G G F E L E L R G D V E M K G K G K V I R T Y W L L G E R - G S S T A G

Ligand-dependent activation of the cytoplasmic guanylate cyclase domains of the human ANP-B and ANP-A receptors was examined in transient expression whole-cell stimulation assays. Cells expressing the ANP-A receptor responded in the same way to stimulation by either α -ANP or pBNP, with a 1.5- to 2-fold increase in cGMP produced over control transfected cells (Fig. 3, *a* and *c*). Cells expressing the ANP-B receptor responded to α -ANP stimulation with a threefold increase in cGMP synthesized as compared to control transfected cells.

Screening of a variety of high complexity λ gt10 cDNA libraries for ANP-B receptor (Fig. 1, legend) and ANP-A receptor⁵ cDNA clones suggests differences in the tissue distribution of the mRNA for these two receptors. Whereas only ANP-A receptor cDNAs were cloned from a human kidney cDNA library¹⁹ (complexity: 5×10^5 clones), cDNAs for both receptors were cloned from human pituitary²⁰ and placental²¹ cDNA libraries. The isolation of only ANP-B receptor cDNAs from human fetal brain²² (complexity: 1.5×10^6 clones), and porcine atrium²³ (complexity: 2.5×10^6 clones) cDNA libraries suggests that the ANP-B receptor is the predominant natriuretic peptide-receptor guanylate cyclase in these two tissues. The isolation of a rat ANP-A receptor cDNA from a rat-brain library⁶ would seem to indicate that this receptor is also present in the brain. Alternatively, this finding may represent a difference between human and rat for ANP-A receptor distribution. *In situ* hybridization with natriuretic peptide-receptor cDNAs should resolve the species-specific differences in expression of these receptors.

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TABLE 1 Particulate guanylate cyclase activity and natriuretic peptide binding of transfected COS-7 cells

Expression vector	Guanylate cyclase activity*	Specific binding†	
		¹²⁵ I-labelled α -ANP	¹²⁵ I-labelled pBNP
Control	0.33 $\pm$ 0.01	3,240 $\pm$ 223	1,087 $\pm$ 164
pRK-ANP-BR	3.50 $\pm$ 0.01	10,694 $\pm$ 1,412	3,643 $\pm$ 26
pRK-ANP-AR	1.91 $\pm$ 0.01	10,313 $\pm$ 893	5,721 $\pm$ 1,107

COS-7 cells maintained in Dulbecco's modified Eagles' medium supplemented with 10% FCS were seeded at 5×10^6 cells per 100 mm plate (guanylate cyclase assay) or 1×10^5 per 35 mm plate (binding assay). Twelve hours later, cells were transfected with control vector pRK, the ANP-B receptor expression vector, or the ANP-A receptor expression vector⁵ ($1 \mu\text{g ml}^{-1}$) using DEAE-dextran. The vector pRK-ANP-BR contained nucleotides 253 to 3,470 (Fig. 1) of the ANP-B receptor in the expression vector pRK5 (R. Klein and D.V.G., unpublished results) under the control of the cytomegalovirus immediate early promoter.

* After transfection (48 h) cells were washed with PBS, scraped into 4 ml PBS and homogenized with 40 strokes of a Dounce homogenizer at 0 °C. Nuclei were pelleted and the supernatant was centrifuged at 100,000g for 30 min in a SW 55-Ti rotor to obtain a membrane pellet. Membrane proteins were solubilized by incubation on ice for 10 min in solution containing 20 mM HEPES pH 7.4, 100 mM NaCl, 10% glycerol, 1% Triton X-100 and 1 mM DTT. Protein concentrations were measured by a dye-binding assay (BioRad). Guanylate cyclase reactions contained 100 μg membrane proteins in 100 μl 20 mM HEPES, pH 7.4, 30 units of creatine phosphokinase, 1 mM GTP, 4 mM MnCl_2 and 0.5 mM isobutyl methylxanthine. After incubation at 37 °C for 10 min, the reaction was stopped by addition of 50 mM acetic acid (500 μl , pH 6.2) and boiled for 3 min. Aliquots were analysed for cGMP after acetylation with 1/20 volume of acetic anhydride/triethylamine (1:2) then radioimmunoassay (Biomedical Technologies). Guanylate cyclase activity is given in pmol cGMP per min per mg protein. Results are expressed as the mean $\pm$ 1 s.d. of triplicate determinations.

† After transfection (48 h) cells were washed twice with buffer A (PBS with 0.2% BSA and 0.1% bacitracin) and incubated with 0.5 nM ¹²⁵I-labelled α -ANP (1,800 Ci mmol⁻¹, Amersham) or ¹²⁵I-labelled pBNP (600 Ci mmol⁻¹) in 2 ml buffer A at room temperature for 1 h. After the incubation, cells were rinsed with cold PBS, and dissolved in 0.2 M NaOH (2 ml) and radioactivity was measured in gamma counter. All measurements were in triplicate. Background binding was measured by incubating the cells with ¹²⁵I-labelled α -ANP or ¹²⁵I-labelled pBNP in the presence of 1 μM α -ANP or pBNP, respectively. Specific binding is given in c.p.m. and was calculated by subtracting the background binding from the total binding.

messenger cGMP production, together with the apparent specific expression of the ANP-B receptor in some tissues, suggests differential multifactorial control of hypotensive functions. In addition, the expression of BNP and α -ANP may each be subject to different forms of control¹⁸. The isolation of ANP-B receptor cDNAs exclusively from a human brain cDNA library suggests that this receptor may be the main mediator of natriuretic peptide action in the central nervous system (CNS). BNP is more widely distributed in the rat CNS than α -ANP, with distinct and non-overlapping CNS distribution for these hormones²⁵. These observations suggest neuromodulatory functions for BNP beyond the central control of cardiovascular homeostasis²⁵.

The teleological reasons for different natriuretic peptide/receptor systems in the CNS and some peripheral tissues are not apparent, given the role of cGMP as a second messenger of natriuretic peptide action¹. According to this hypothesis the differential action of natriuretic peptides in target tissues would be through the expression of different cGMP targets such as a cGMP-gated kidney cation channel²⁶, a cGMP-regulated phosphodiesterase²⁷ and the activation of cGMP-dependent protein kinase²⁸, together with the tissue-specific expression of kinase substrates. As well as expressing different tissue responses to natriuretic peptides, the ANP-B and ANP-A receptors could be subject to different patterns of regulation, or they may each

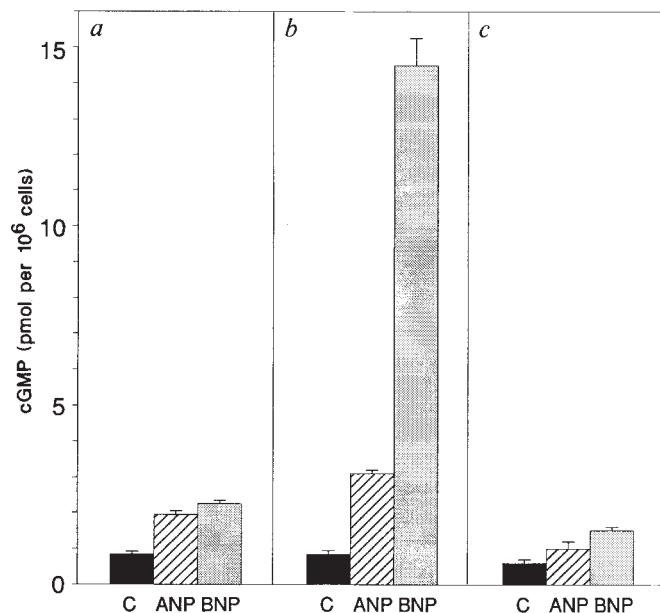


FIG. 3 Stimulation of cGMP production by natriuretic peptide. COS-7 cells transfected with human-ANP-A-receptor expression vector⁵ (panel a), human-ANP-B-receptor expression vector (panel b) or pRK (control; panel c), were either not treated (C) or stimulated with 500 nM porcine BNP (BNP) or human α -ANP (ANP) for 5 min. Results are expressed as the mean amount of cGMP (pmol) per culture $\pm$ 1 s.d. for 3 independent determinations.

METHODS. COS-7 cells were seeded at a density of 1×10^6 cells per well in a 60 mm plate with high glucose DMEM plus 10% heat-inactivated dialysed calf serum and maintained for 12 h at 37 °C in 7% CO₂, 93% air. Plasmid DNA (3 μg) was transfected with 400 $\mu\text{g ml}^{-1}$ of DEAE-dextran in high glucose DMEM (2 ml) + 10% Nuserum (Gibco) for 4 h. Cells were shocked with 20% glycerol in PBS for 1 min, fresh medium was added and cells were maintained for 72 h before stimulation. Non-treated cultures were incubated at 37 °C in DMEM with 25 mM HEPES pH 7.2, 0.1 mM isobutyl methylxanthine at 37 °C for 10 min, medium was aspirated and 6% trichloroacetic acid was added. Stimulated samples were treated for an additional 5 min in the presence of 500 nM peptide before trichloroacetic acid treatment. Samples were frozen at -20 °C for 1 h, thawed at room temperature, and cell debris was removed by centrifugation at 2,500g for 10 min. Samples were extracted once with 4 volumes of water-saturated ether, briefly evaporated and assayed for cGMP as described in Table 1.

have additional different signal-transduction pathways which are biochemically distinct from cGMP as a second messenger. These possibilities may be reflected in the less conserved (63% identity) kinase homology region, as opposed to the highly conserved (88% identity) cyclase homology region of these two natriuretic-peptide receptors. □

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Exocrinopathy resembling Sjögren's syndrome in HTLV-1 *tax* transgenic mice

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HUMAN T-cell leukaemia virus 1 (HTLV-1) is a retrovirus aetiologically associated with adult T-cell leukaemia (ATL)¹⁻³, tropical spastic paraparesis (TSP)⁴⁻⁶ and possibly multiple sclerosis (MS)⁷ in humans. Three founder lines of transgenic mice containing the HTLV-1 *tax* gene under the control of the viral long terminal repeat (LTR)⁸ have previously been shown to develop neurofibromas⁹. Further analysis of these animals has now revealed that they also develop an exocrinopathy involving the salivary and lachrymal glands. This pathology resembles Sjögren's syndrome, a disease of presumed autoimmune aetiology, features of which are sometimes reported in HTLV-1 associated conditions. Mice with an HTLV-1 *tax* transgene might be a useful model for studying the development of Sjögren-syndrome-like pathology.

Within several weeks after birth of mice containing the HTLV-1 *tax* gene, proliferation of ductal epithelial cells is noted in the submandibular, parotid and minor salivary glands (Fig. 1b, d). This proliferation is multifocal and occurs diffusely throughout the gland. In the early stages architecture of the acinus remains well preserved and no lymphocytic infiltration is observed, but over the course of several weeks, the ductal proliferation dramatically progresses and the structure is distorted. Ductal proliferation to a lesser degree is also observed in the sublingual gland (Fig. 1c), but generally after significant ductal proliferation has occurred in the submandibular and parotid glands. Ductal proliferation also occurs in the accessory salivary glands within the oral cavity of the transgenic mice (Fig. 1d).

Lymphocytic infiltration of the salivary glands occurs after there has been significant proliferation of ductal epithelium. Initially, lymphocytes are found adjacent to the proliferating nests of epithelial cells. As the proliferation progresses, lymphocytes as well as plasma cells infiltrate and surround the masses of proliferating cells as well as adjacent salivary gland acini (Fig. 1e). Mice surviving six to eight months exhibit extensive epithelial island enlargement with lymphocytic infiltration, leading to destruction of acinus architecture, and hyalinization and thickening of basement membranes (Fig. 1f).

Electron-microscopic analysis of the proliferating ductal cells

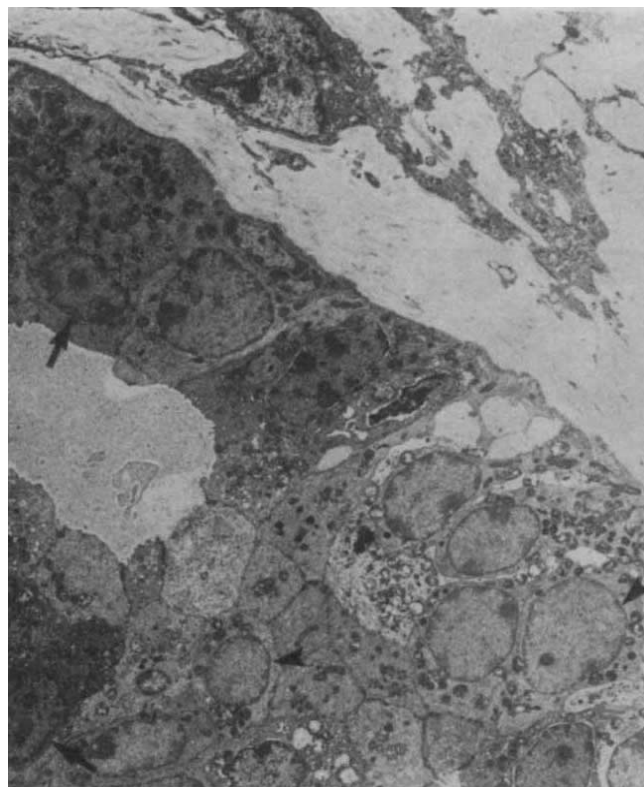


FIG. 2 Electron micrograph of early submandibular ductal proliferation in the 6-2 line. Arrows, nuclei of normal ductal cells; arrowheads, nuclei of abnormal ductal cells. Magnification $\times 2,400$. Tissue preparation as previously described²⁴.

in the salivary gland shows junctional complexes and external lamina without myofilaments consistent with these cells being of an epithelial origin. The nuclei of the proliferating cells exhibit an open nuclear pattern with peripherally clumped chromatin (Fig. 2).

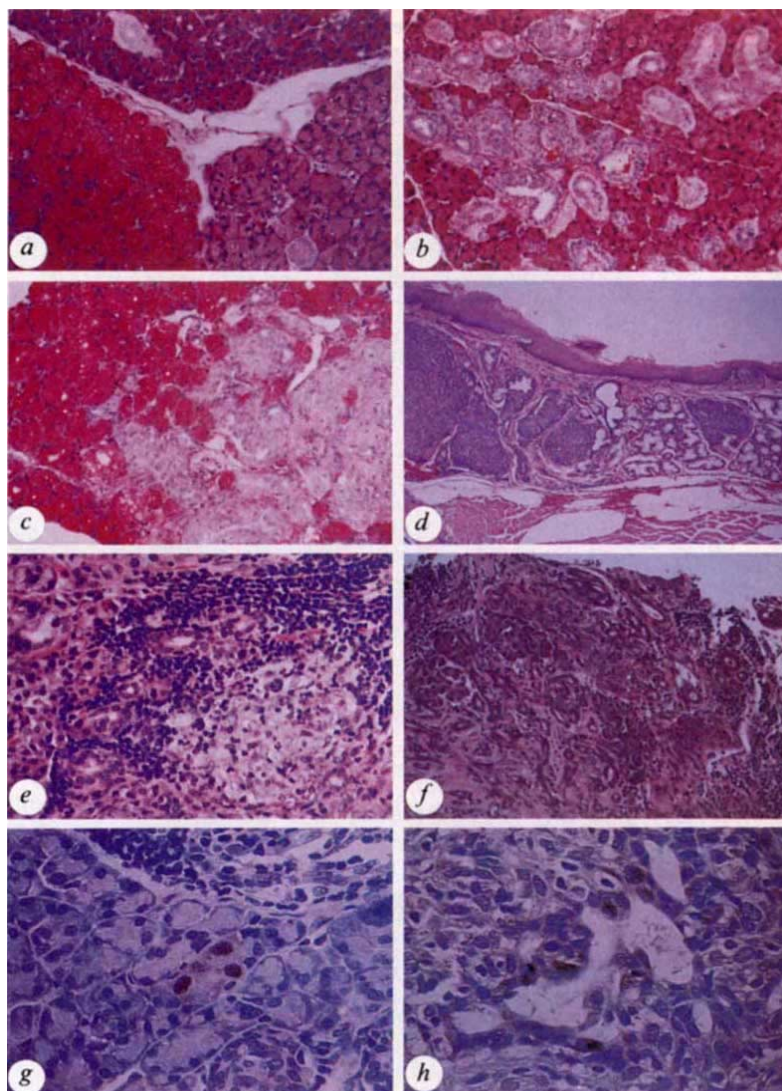
Nests of proliferating cells are also seen in the lachrymal and harderian glands of these animals, although this tends to occur late and is not as extensive as the proliferation found in the salivary glands.

The degree of salivary-gland pathology in these transgenic mice corresponds to the level of expression of the *tax* gene in the ductal epithelial cells as seen by western blot analysis of tissue extracts. No Tax protein was detected in control-mouse tissues (Fig. 3, lanes 1, 2) but was found in salivary glands of each founder line including lines 6-2, 8-4 and 12-2 (Fig. 3, lanes 3-8). Both male and female adult animals produce equal amounts of Tax protein (Fig. 3, lanes 3-6). Low levels of Tax were measured in 5-week-old mice who exhibited early pathological changes. Tax levels increased progressively three- to fivefold as the mice matured and correlated with the increasing severity of pathological change. Immunocytochemistry performed on tissue sections demonstrated that the Tax protein was localized predominantly in the nucleus (Fig. 1g) and particularly in ductal cells during the early stages of the disease (Fig. 1h).

The changes occurring in the major and minor salivary glands as well as lachrymal glands resemble features observed in Sjögren's syndrome. Sjögren's syndrome is an exocrinopathy of presumed autoimmune aetiology producing kerato-conjunctivitis sicca (dry eyes) and xerostomia (dry mouth) with a characteristic sialadenitis involving lymphocytic infiltration of the salivary and lachrymal glands¹⁰⁻¹² as well as proliferating nests of epithelial cells. Ultimately, the acinus is destroyed with deposition of hyalin material.

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FIG. 1 Microscopic examination of salivary glands from normal (a) and HTLV-1-*tax* transgenic mice (b-h). Founder series 6-2: b, c, g, h; series 12-1: d-f. a, Normal adult sublingual, submandibular and parotid glands; b, submandibular gland, 5 weeks old; c, sublingual gland, 11 weeks old; d, minor salivary glands of oral cavity, 16 weeks old; e, submandibular gland, 20 weeks old; f, submandibular gland, 24 weeks old; g, submandibular gland, 16 weeks old and h, submandibular gland, 16 weeks old. Paraffin-embedded sections of salivary glands, stained by periodic acid-Schiff (a, b and c) or haematoxylin and eosin (d, e, f). Immunocytochemistry using anti-*tax* antibody was performed by standard method²³ (g and h). Magnifications: $\times 60$ (a-c, f), $\times 24$ (d), $\times 150$ (e), $\times 240$ (g, h).



Sjögren's syndrome has been associated with a spectrum of lymphoproliferative disorders ranging from benign hyperplasia to overt lymphoma¹³. These transgenic mice also develop marked enlargement of the lymph nodes within and adjacent to the salivary glands. Histologically, this represents significant hyperplasia of the medullary and cortical regions of the lymph nodes as well as plasma cell infiltration. We have not, however, observed the occurrence of a lymphoma in these mice.

An association between HTLV-1 and Sjögren's syndrome has been suggested indirectly through case reports of HTLV-1 infected patients with tropical spastic paraparesis who developed features of Sjögren's syndrome^{4,14,15}. Features of Sjögren's syndrome have been reported in intravenous drug abusers¹⁶ who are at high risk of HTLV-1 infection¹⁷.

Although several case reports have described the occurrence of salivary-gland lesions in HIV infected patients¹⁸⁻²⁰, these studies did not address whether these individuals were also infected with HTLV-1. Epidemiological data suggest that a high proportion of HIV-infected patients are also infected with HTLV-1 (ref. 17).

This transgenic model suggests that HTLV-1 is tropic for ductal epithelium of the salivary and lachrymal glands and produces features which resemble Sjögren's syndrome. The primary event in the development of the exocrinopathy may be a virally induced proliferation and perturbation of the function of ductal epithelium, followed by a lymphocytic response. This sequence of events differs from that proposed by currently

accepted models of Sjögren's syndrome pathogenesis, where lymphocytic infiltration is thought to precede ductal cell proliferation^{21,22}. Our transgenic model may provide new insights into the study of autoimmune disorders and the pathogenesis of

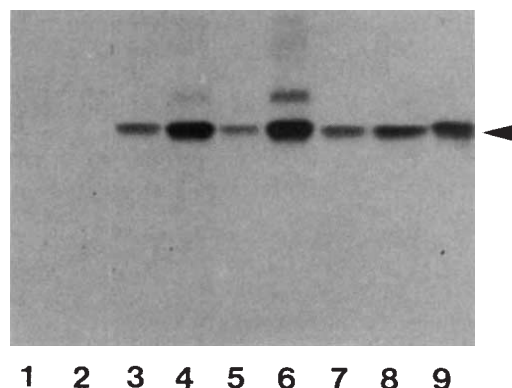


FIG. 3 Protein immunoblot analysis. Lane 1, normal salivary gland; lane 2, normal muscle; lanes 3 and 4, salivary gland and muscle, 6-2-97 male; lanes 5 and 6, salivary gland and muscle, 6-2-98 female; lane 7 and 8, salivary gland and muscle, 12-2-45 male; lane 9, neurofibroma cell line PX-1 derived from a 6-2 mouse. Arrowhead indicates the 40K Tax protein. 80 μ g of total protein was loaded per lane and analysed as previously described^{8,25}.

HTLV-1-induced proliferative diseases induced by the transactivating Tax protein. □

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Changing Fos oncoprotein to a Jun-independent DNA-binding protein with GCN4 dimerization specificity by swapping 'leucine zippers'

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A STRUCTURAL motif for DNA-binding proteins, the 'leucine zipper', has been proposed for the *jun*, *fos* and *myc* gene products, the yeast transcriptional activator GCN4, and the C/EBP enhancer-binding protein¹. These proteins all contain a region with four or five leucine residues spaced exactly seven amino acid residues apart whose sequence is consistent with the formation of an amphipathic α -helix. It has been proposed that the leucine zipper consists of two interdigitated α -helices, one from each monomer, that constitute the dimerization function necessary for high-affinity binding to DNA; an adjacent region of basic residues is thought to be responsible for specific protein-DNA contacts¹. In support of this model, substitution of the leucine residues within the motif can abolish dimerization and DNA-binding²⁻⁶, and a synthetic peptide corresponding to the GCN4 leucine zipper forms α -helical dimers⁷. Despite the conserved leucine residues, however, each protein has a distinct dimerization specificity. Specifically, GCN4 homodimer, Jun homodimer and Fos-Jun heterodimer proteins bind to the same DNA site, whereas Fos is unable to form homodimers, bind DNA, or interact with GCN4 (refs 8-14). Here, we alter the dimerization specificity of Fos by precisely replacing its leucine zipper with that from GCN4. This Fos-GCN4 chimaeric protein is able to bind to the target site in the absence of Jun, and can form DNA-binding heterodimers with GCN4 but not with Jun. These results indicate that the leucine zipper is sufficient to confer dimerization specificity and strongly suggest that Fos contacts DNA directly.

The products of the nuclear proto-oncogenes *fos* and *jun* form a heterodimeric complex which binds to the transcriptional regulatory element known as the AP-1 site^{2,4-6,12-14}. Yeast GCN4 protein binds as a homodimer to target sequences that are indistinguishable from AP-1 sites^{8,9}. Extensive analysis indicates that the optimal GCN4 binding site is dyad-symmetric and that each GCN4 monomer directly contacts a half-site^{15,16}. By analogy, each subunit of a Fos-Jun heterodimer would be expected to contact a half-site. This idea has been supported by the finding that Fos, Jun, and GCN4 contain a conserved stretch of basic residues equally spaced from their leucine zippers, and mutations in the Fos and Jun basic region prevent DNA-binding, but not dimerization^{2,4-6}. Although the failure of Fos to bind DNA could reflect its inability to form homodimers, its contribution to specific DNA-binding could also be by an indirect effect mediated through Jun.

To determine whether the leucine zipper confers dimerization specificity and Fos has the potential to contact DNA, we created Fos^G, a chimaeric protein in which Fos residues 23-160 (including the basic region) are fused to the carboxy-terminal 33 amino acids of GCN4 (containing the leucine zipper, but unable to bind DNA; see ref. 17) (Fig. 1). The spacing of the leucine zipper and the basic domain in Fos^G is identical to that in Fos and GCN4. The LexA domain at the amino-terminus of the protein lacks the dimerization function^{18,19} and is irrelevant to these experiments (see below).

To test the DNA-binding properties of Fos^G, ³⁵S-labelled Fos^G was synthesized *in vitro*, incubated with the optimal GCN4/AP-1 site (ATGACTCAT), and electrophoresed in non-denaturing polyacrylamide gels (Fig. 2a). A band is seen which is dependent on the addition of both the protein and DNA, indicating that Fos^G can bind to this DNA fragment. We tested the specificity of binding with a DNA containing a point mutation, AGGACTCAT; this mutation leads to a significant reduction in binding by GCN4 and by the Jun-Fos complex^{9,10,15}. Complex formation by Fos^G was also greatly reduced by this point mutation, indicating that the chimaeric protein has similar DNA sequence recognition properties to those of GCN4 and the Jun-Fos complex.

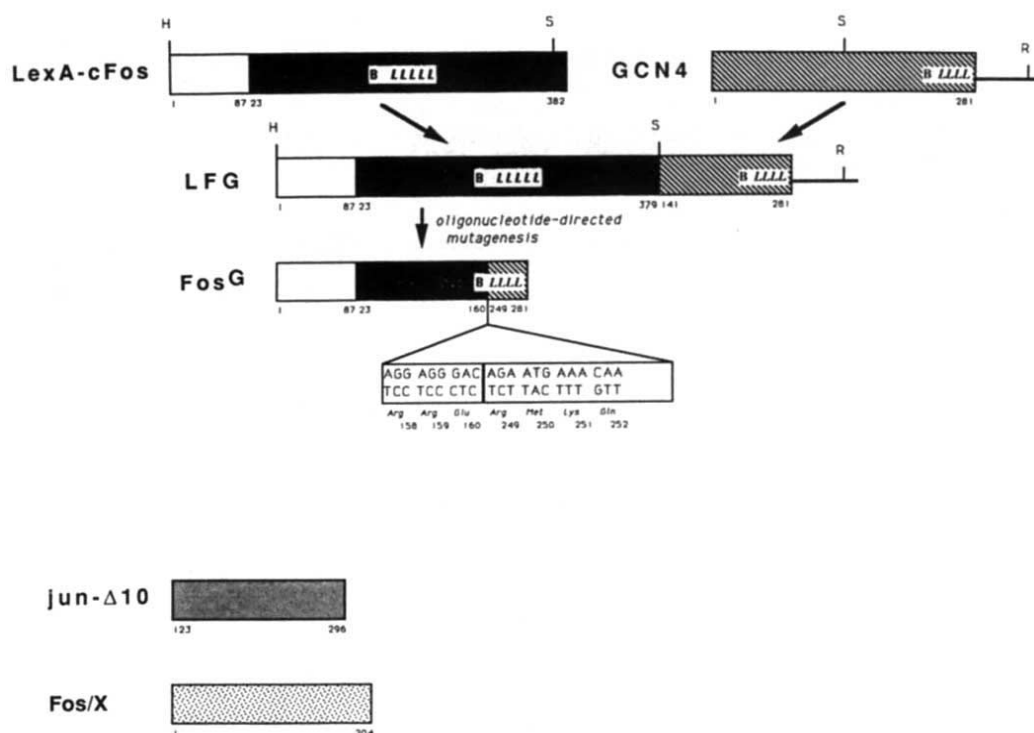
The fact that Fos^G binds to the dyad-symmetric GCN4/AP-1 site suggests that it can form homodimers, presumably mediated by the GCN4 leucine zipper. If the leucine zipper confers dimerization specificity, then Fos^G should form heterodimers with GCN4 but not with Jun. Indeed, when we synthesized GCN4 and Fos^G together and incubated them with the target DNA, we observed a new protein-DNA complex with an intermediate mobility (Fig. 2b) indicative of heterodimer formation. The Fos^G-GCN4 heterodimer and the GCN4 homodimer bands were of equal intensity, but the presumed Fos^G homodimer band was much fainter. This observation could reflect a decrease in either the dimerization ability or in the affinity for target DNA of the Fos^G homodimer compared with the heterodimer; we favour the latter explanation. In any event, our results exclude the possibility that dimerization by the LexA domain influences binding by Fos^G, because although the heterodimer contains only one LexA moiety, it functions more effectively than the homodimer.

Additional observations indicate that Fos^G is a derivative of Fos with an altered dimerization specificity (Fig. 2c). First, when Fos^G and a Jun derivative were co-synthesized, only the Fos^G complex was seen, indicating that Fos^G and Jun cannot form heterodimers. The failure to observe new bands was not due to the co-migration of heterodimer and homodimer complexes, because the relative molecular masses of Fos^G and the Jun derivative differ (under our conditions, the Jun homodimer complex cannot be seen¹³). Second, unlike Fos^G, a Fos derivative does not bind DNA or form a heterodimer complex after co-synthesis with GCN4, but it will form a DNA-binding complex when co-synthesized with Jun (refs 2 and 12-14; data not shown).

FIG. 1 Structure of proteins.

Top line, structure of LexA-c-Fos, which is composed of the LexA DNA-binding domain (open box) fused to the Fos oncoprotein (black box), and the structure of GCN4 (hatched box). Leucine zippers, L; adjacent basic regions, B; H, R and S indicate restriction sites (H, *Hind*III site; S, *Sal*I site; R, *Eco*RI site) used in the cloning of LFG, which is shown in the second line from the top. (The wild-type *GCN4* gene does not contain a *Sal*I site; the site shown occurs in *LexA-gcn4-Δ29* (ref. 20), a deleted derivative used in the construction of LFG.) Shown below LFG is Fos^G, with the sequence of the junction between Fos^G and GCN4 indicated. Jun-Δ10 is a deleted Jun derivative. The Fos derivative, Fos/X, was obtained from T. Halazonetis¹³. Numbers below protein structures indicate amino-acid residues. The thick line represents non-coding sequences.

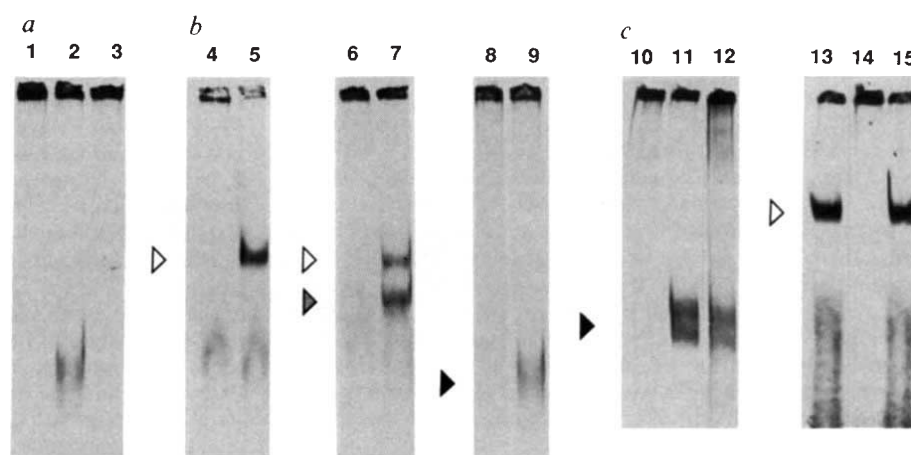
METHODS. The DNA LFG was constructed by joining the *Hind*III-*Sal*I fragment of the gene encoding LexA-c-Fos (ref. 21) with the *Sal*I-*Eco*RI fragment of *GCN4* (the *Eco*RI site is downstream of the structural gene) and cloning the resulting fusion into *Hind*III/*Eco*RI-digested YCm90, a vector derived from YCp88 (ref. 17) that contain an SP6 promoter and an M13 origin of replication.



The DNA molecule encoding the chimaeric Fos^G protein was generated from LFG by oligonucleotide-directed mutagenesis using uracil-substituted single-stranded templates²²; the junction between *fos* and *GCN4* was confirmed by sequencing. The *jun* derivative, *jun-Δ10* was generated by cloning the *Sal*I-*Eco*RI fragment of *LexA-jun-Δ5* (ref. 23) into pSP64, a vector containing the SP6 promoter.

FIG. 2 Specificity of complex formation.

a. ³⁵S-labelled, *in vitro*-translated Fos^G protein was tested for ability to bind to the GCN4/AP-1 site in the absence of DNA (lane 1), in the presence of a 631-base pair DNA fragment containing the optimal GCN4/AP-1 site ATGACTCAT (lane 2), or in the presence of a similar fragment containing the point mutation AGGACTCAT (lane 3). A DNA-protein complex is observed only in the reaction containing the optimal GCN4/AP-1 site (lane 2, black arrow). **b.** To test for heterodimer formation, GCN4 (lane 5), Fos^G (lane 9) or a co-synthesis of both proteins (lane 7), were each incubated with the 631-base pair fragment containing the optimal GCN4/AP-1 binding site. When synthesized alone, GCN4 (lane 5, open arrow) and Fos^G (lane 9, black arrow) each form complexes. When synthesized together, a new complex of intermediate mobility is seen (lane 7, stippled arrow; see text). In the absence of DNA, no complexes are evident (lanes 4, 6 and 8). The diffuse band at the bottom of lanes 4 and 5 represents a minor artefactual translation product made only in syntheses of full-length GCN4 protein; it is unrelated to any DNA-binding phenomenon because it is found in the absence of added DNA (lane 4; refs 8 and 17). Because this artefactual band is associated only with synthesis of full-length GCN4, it does not interfere with the results, except in lane 7 where it obscures the Fos^G complex band due to almost similar electrophoretic gel mobilities; **c.** To test for specificity of dimerization, Jun (lane 10), Fos^G (lane 11) or a co-synthesis of both Jun and Fos^G proteins (lane 12) were incubated with the optimal binding-site-containing fragment. Only Fos^G complexes are seen (lanes 11 and 12, black arrows). When GCN4 protein (lane 13) or a co-synthesis of GCN4 and c-Fos proteins (lane 15) are incubated with the target DNA, only GCN4 complexes are found. No complex was observed when c-Fos protein alone was incubated with the target DNA (lane 14). The



Jun and c-Fos proteins synthesized in these experiments were functional; as expected¹²⁻¹⁴, when both proteins are mixed and incubated with the DNA fragment containing the optimal GCN4/AP-1 site, they generate a protein-DNA complex (data not shown). The experiments represented in the separate panels involve different protein preparations, gels and autoradiograph exposure, which accounts for any small differences in the Fos^G complex band intensity between panels.

METHODS. Labelling of protein and analysis of DNA-protein complexes have been described^{8,17}, except that the binding buffer contained poly(dI · dC) at a final concentration of 100 μg ml⁻¹ instead of salmon sperm DNA. Cellular-Fos protein was generated from pGEMFos X (from T. Halazonetis). Yields and purity of protein preparations were routinely established by SDS-PAGE and autoradiography.

An important conclusion from our results is that the leucine zipper is sufficient to confer dimerization specificity. Specifically, Fos^G contains only the 33 C-terminal residues of GCN4 (the leucine zipper region) but, unlike Fos, can form heterodimers with GCN4 but not with Jun. Moreover, these observations together with the fact that Fos^G alone binds the GCN4/AP-1 site strongly suggest that the leucine zipper is sufficient for dimerization *per se*. (The possibility that other regions of Fos and GCN4 contribute a common, non-specific dimerization function cannot be excluded, but seems unlikely.) These results agree with an earlier observation that a synthetic peptide containing the 33 C-terminal residues of GCN4 forms stable dimers⁷: the conditions here were more physiological, however (concentrations 10⁴–10⁵ times lower) and experiments were performed in the context of an intact DNA-binding domain.

The leucine-zipper motif was initially defined by the presence of four to five leucine residues spaced seven amino-acid residues apart in a region of the protein that could permit the formation of an α -helix; other than this, there was little overall amino-acid sequence similarity¹. The fact that swapping the leucine zipper of Fos for that of GCN4 yields a specific DNA-binding protein indicates that these regions are functionally homologous. Nevertheless, it is clear that although the conserved leucines are important for the dimerization of C/EBP (ref. 3), Fos^{2,4,6}, Jun^{4,5}, and GCN4 (J.W.S., W. J. van Heeckeren and K.S., unpublished results), other non-conserved residues in the various zipper regions must be involved in dimerization specificity. Thus, the ability of this class of proteins to form homodimers or heterodimers will depend on the association properties of individual leucine-zipper regions.

Another conclusion from our experiments is that Fos has an inherent specific DNA-binding activity. Because the GCN4 leucine-zipper region alone does not bind DNA¹⁷, some region of Fos (probably the basic region) must contribute to the ability of Fos^G to interact directly with specific DNA sequences. Furthermore, because the GCN4 leucine zipper can convert Fos into a DNA-binding protein that bypasses the requirement for Jun, the failure of native Fos to bind to DNA almost certainly reflects its inability to form homodimers. In terms of the Fos-Jun heterodimer, these considerations strongly argue against the model that Fos indirectly affects DNA-binding by Jun. Instead, they indicate that Fos and Jun monomers in the heteromeric complex interact with adjacent half-sites.

Finally, chimaeric proteins such as Fos^G should be useful in elucidating the function *in vivo* of proteins involved in heteromeric complexes. For example, transcription involving the AP-1 site is regulated by phorbol esters^{10,11}, a process which might be mediated by Jun and/or Fos. As Fos^G derivatives can bind to the AP-1 site without Jun, it should be possible to separate the effects of these two oncoproteins. Chimaeric proteins containing the GCN4 leucine zipper could be used to investigate how proteins with leucine zippers might bind DNA specifically. For example, specific DNA-binding by the Myc oncoprotein has yet to be demonstrated, but perhaps by replacing its leucine zipper with that from GCN4, Myc could be converted to a DNA-binding protein whose target sites might be identified by the random selection method¹⁶. □

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Alternative production of calcitonin and CGRP mRNA is regulated at the calcitonin-specific splice acceptor

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ALTERNATIVE splicing of eukaryotic messenger RNA precursors represents a common mechanism for generating multiple transcripts from a single gene^{1,2}. Although there has been increasing information concerning the sequence requirements and the biochemical mechanisms involved in the constitutive splicing of primary RNA transcripts, very little is known about the sequences or mechanisms which determine alternative RNA-processing events in complex transcription units^{3–6}. The calcitonin/calcitonin gene-related peptide (CGRP) primary RNA transcript undergoes tissue-specific alternative processing, resulting in the differential production of calcitonin mRNA in thyroid C cells and CGRP mRNA in neurons of the central and peripheral nervous systems^{7,8}. To elucidate the molecular mechanisms underlying these alternative RNA processing events, we have examined the nucleotide sequences involved in the production of calcitonin and CGRP mRNAs. Analyses of HeLa and F9 cell lines transfected with a variety of mutant calcitonin/CGRP transcription units have demonstrated that alternative splice-site selection is primarily regulated by *cis*-active element(s) near the calcitonin-specific 3'-splice junction. We suggest that the tissue-specific pattern of alternative RNA processing is conferred by sequence information at the calcitonin-specific acceptor which serves to inhibit the production of calcitonin transcripts in CGRP-producing cells.

The rat calcitonin/CGRP gene is comprised of six exons; calcitonin mRNA is produced by splicing of the first three exons to the fourth exon, accompanied by cleavage and polyadenylation at the 3' end of the fourth exon. CGRP mRNA production results from the splicing of the first three exons to the fifth and sixth exons and use of a distal poly(A) site at the 3' end of the sixth exon (Fig. 1a)^{7,9}. Previous studies on the expression of a metallothionein-calcitonin/CGRP fusion gene in transgenic mice demonstrated that almost all tissues produced predominantly calcitonin mRNA¹⁰. In most neurons however, CGRP transcripts represented the main RNA species. The simplest interpretation of these results is that the ability to splice the calcitonin/CGRP primary transcript to produce mature CGRP mRNA requires a neuron-specific splicing machinery, thereby suggesting that calcitonin mRNA is likely to represent the unregulated splicing choice. Alternatively, production of calcitonin-specific transcripts could require a tissue-specific factor which is widely distributed, but absent from neuronal tissues.

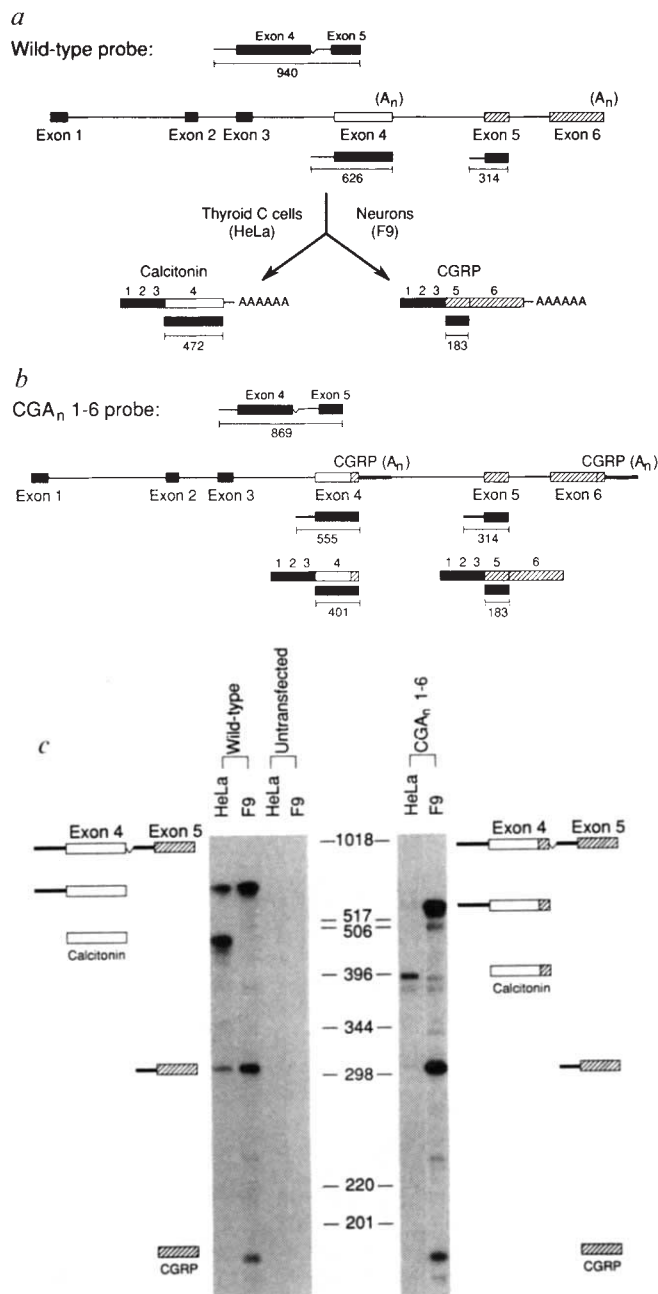
Two potential mechanisms have been proposed for the regulation of alternative splice-site use in the calcitonin/CGRP gene. One mechanism involves sequence or site-specific poly(A) site

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FIG. 1 Replacement of the calcitonin poly(A) site does not alter differential processing of the calcitonin/CGRP transcript. *a*, Schematic representation of the rat calcitonin/CGRP primary transcript demonstrating differential RNA processing in neurons and thyroid C cells. A wild-type RNA probe for ribonuclease protection analysis containing adjacent intron/exon sequences at the calcitonin (exon 4) and CGRP-specific (exon 5) splice junctions is shown; structures and sizes of predicted RNase protection fragments are indicated. *b*, A mutant transcription unit (CGA_n 1-6) in which the calcitonin poly(A) site (-140 to +54 relative to the 3' end of the fourth exon) was replaced with a fragment containing the CGRP poly(A) site (-69 to +256 relative to the 3' end of the sixth exon) is shown; the structures and sizes of predicted RNase protection fragments are schematically indicated. *c*, RNase protection analyses of RNA from untransfected and HeLa or F9 permanent transfectants expressing either the wild-type or CGA_n 1-6 calcitonin/CGRP transcription units are shown; structures and migration positions of predicted RNase protection fragments and radiolabelled DNA markers (1 kb ladder; BRL) are indicated.

METHODS. Permanently transfected cell lines were established using calcium phosphate co-precipitation with plasmids containing the neomycin resistance gene and the calcitonin/CGRP transcription unit as previously described^{11,25}. Polyclonal populations of G418-resistant clones were selected and RNA was prepared by the guanidinium-isothiocyanate method²⁶. For construction of the wild-type RNase protection probe, a 154-bp region from the 3' end of the third intron and the complete calcitonin-specific exon (exon 4) was fused to sequences corresponding to 131 bp from the 3' end of the fourth intron and the complete CGRP-specific exon (exon 5). Construction of an RNase protection probe specific for the CGA_n 1-6 transcription unit was made in a parallel manner. DNA fragments were obtained by amplification of specific genomic sequences from the wild-type and mutant transcription units using the polymerase chain reaction (PCR) with *Taq* DNA polymerase (Perkin-Elmer/Cetus) and synthetic oligonucleotides according to manufacturer's instructions. Amplified DNA fragments were then cloned into a transcription vector (pBKS II; Stratagene), linearized with *Hind*III and anti-sense RNA was transcribed (specific activity = 4×10^8 c.p.m. μg^{-1}) using T7 RNA polymerase in the presence of [α -³²P]UTP. Ribonuclease protection assays were performed essentially as described²⁷; total cellular RNA (1–10 μg) was hybridized to 5×10^5 c.p.m. of a labelled antisense RNA probe at 45 °C for 14–18 h. Ribonuclease A digestion (20 $\mu\text{g ml}^{-1}$) was carried out at 30 °C for 45 min. Protected RNA fragments were separated by size on a 4% acrylamide/7 M urea gel as previously described²⁷. Densitometric analyses of RNase protection autoradiograms were performed using an LKB Ultrosan XL densitometer. Quantitation of the relative levels of specific RNA species was calculated by correcting integrated densitometric peak areas for the number of uridine residues present in each protected fragment.



selection, and another proposes that choice of the 3' (acceptor) splice-site is the critically regulated event. In order to investigate such mechanisms, we have developed model systems in which transfected cell lines exhibit mRNA-processing choices analogous to those observed *in vivo*¹¹. A ribonuclease protection assay was used to quantitate accurately alternative splice-site use in such cell lines (Fig. 1*a*). Human epithelial (HeLa) and mouse teratocarcinoma (F9) cell lines, permanently transfected with the wild-type rat calcitonin/CGRP gene, paralleled the alternative RNA processing events demonstrated by thyroid C cells (>98% calcitonin mRNA) and neurons (>94% CGRP mRNA), respectively (Fig. 1*a*, *c*). Both cell lines also generated protected fragments corresponding to RNA species in which cell-specific RNA processing had not occurred. Hybridization analyses to size-fractionated RNA (data not shown) confirmed the cell-specific alternative RNA processing events and demonstrated the accumulation of significant levels of high molecular weight RNA species previously identified as partially processed nuclear RNA precursors^{11,12}.

Although *in vitro* splicing analyses of calcitonin/CGRP transcripts have suggested that alternative RNA processing may be regulated by cell-specific poly(A) site selection¹³, calcitonin- and CGRP-producing cell lines exhibited no differential poly(A) site utilization when transfected with transcription units in which the calcitonin and CGRP poly(A) sites were placed in tandem array¹¹. To further address this question, a calcitonin/CGRP transcription unit was constructed in which the calcitonin (exon 4) poly(A) site was replaced by sequences containing the CGRP (exon 6) cleavage/polyadenylation signal (Fig. 1*b*). Analyses of mRNA from cell lines transfected with the mutant (CGA_n 1-6) calcitonin/CGRP transcription unit revealed cell-specific splice acceptor use similar to that of the wild-type gene for both cell types (Fig. 1*c*). Replacement of the calcitonin poly(A) site with sequences containing the CGRP cleavage/polyadenylation signal neither increased utilization of the calcitonin-specific splice acceptor in F9 cells (94% CGRP), nor altered calcitonin acceptor use in transfected HeLa cells (99% calcitonin). These experiments strongly suggest that differential

poly(A) site utilization is not the key regulatory event involved in calcitonin/CGRP alternative RNA processing, indicating that tissue-specific processing of this gene is regulated by alternative splice-site selection.

The observation that alternative splicing occurs in a tissue-specific manner has suggested that *trans*-acting factors must interact with the pre-mRNA; however, *cis*-active elements within the transcript must ultimately determine the nature of the final

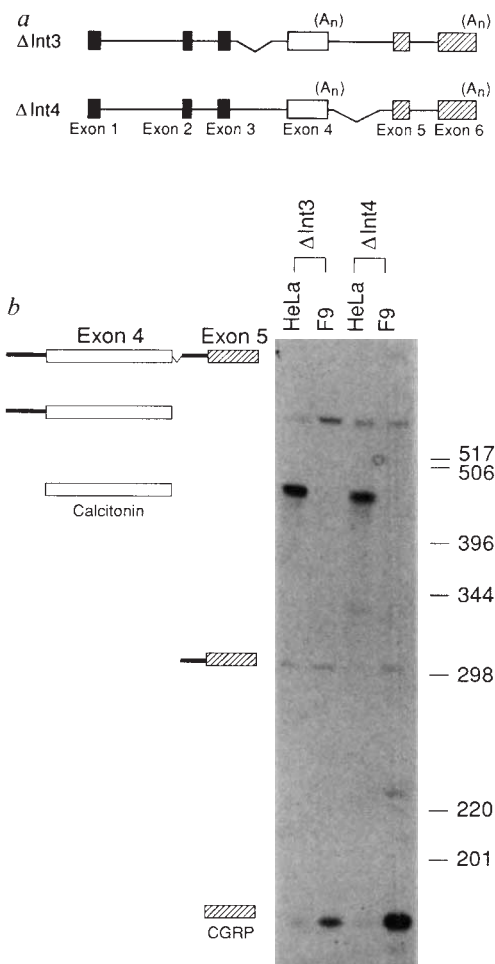


FIG. 2 Large deletions in the third and fourth introns do not affect calcitonin/CGRP alternative processing. *a*, Schematic representations of mutant calcitonin/CGRP transcription units indicating the locations of a 380-bp deletion in the third intron (ΔInt3) and a 590-bp deletion in the fourth intron (ΔInt4). *b*, Ribonuclease protection analyses of RNA from polyclonal permanent transfectants expressing the ΔInt3 and ΔInt4 calcitonin/CGRP transcription units demonstrates maintenance of cell-specific differential RNA processing; the structures and migration positions of predicted RNase protection fragments are indicated. The migration position of radiolabelled DNA markers are designated.

METHODS. Construction of the ΔInt3 transcription unit involved cleavage of the wild-type calcitonin/CGRP gene at a unique *Bst*E II site, 77 bp downstream from the exon 3 splice donor, followed by subsequent digestion with *Bal* 31 exonuclease as previously described²⁷. The extent of the deletion, from the *Bst*E II site to a point 206 bp upstream from the fourth exon, was determined by DNA sequence analysis of double-stranded DNA templates according to the chain-termination method of Sanger *et al.*, using synthetic oligonucleotide primers and T7 DNA polymerase (Sequenase; United States Biochemicals)²⁷. Construction of the ΔInt4 transcription unit was made by introduction of an *Eco*RI restriction site into the fourth intron 119 bp upstream from the CGRP-specific splice acceptor site by oligonucleotide-directed mutagenesis in M13mp18 as previously described²⁸; the deletion extended from a *Bgl*II site, 55 bp downstream from the 3' end of the fourth exon, to the introduced *Eco*RI restriction site. Ribonuclease protection assays, using the wild-type RNase probe, and densitometric analyses were performed as described in Fig. 1.

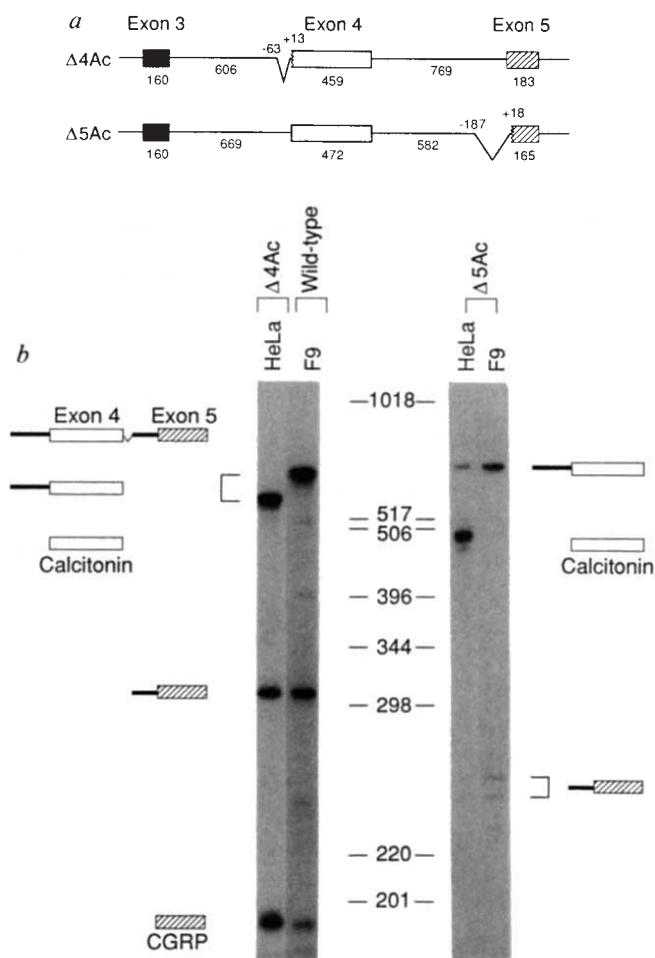


FIG. 3 Exon 4 and exon 5 splice acceptor deletions reveal that regulation of calcitonin/CGRP alternative RNA processing is at the calcitonin-specific splice acceptor. *a*, Schematic diagrams of a portion of mutant calcitonin/CGRP transcription units in which either the calcitonin- ($\Delta 4\text{Ac}$) or CGRP-specific ($\Delta 5\text{Ac}$) splice acceptor regions have been deleted. The coordinates of the deletions, relative to the beginning of either the fourth or fifth exons, and the sizes of adjacent exons and introns are indicated. *b*, Ribonuclease protection analyses of RNA from permanently transfected HeLa and F9 polyclonal cell lines expressing the wild-type, $\Delta 4\text{Ac}$ or $\Delta 5\text{Ac}$ calcitonin/CGRP transcription units; the structures and migration positions of predicted RNase protection fragments and radiolabelled DNA markers are indicated. A doublet representing an RNA species in which CGRP-specific splicing has not occurred (bracketed) is presumed to result from transient denaturation of an A+T-rich region during the assay.

METHODS. Construction of the $\Delta 4\text{Ac}$ calcitonin/CGRP transcription unit was made by introduction of a unique *Kpn*I restriction site, 63 bp upstream of the fourth exon, and a *Clal* restriction site, 13 bp into the fourth exon, by oligonucleotide-directed mutagenesis in M13mp18, as previously described²⁸; deletion of the calcitonin-specific splice acceptor region involved removal of the 77bp *Kpn*I-*Clal* fragment. Construction of the $\Delta 5\text{Ac}$ transcription unit was made by introduction of a *Sma*I restriction site, 187 bp upstream from the fifth exon, and an *Bam*HI site, 18 bp into the fifth exon, by utilization of the polymerase chain reaction (PCR) with synthetic oligonucleotides. The transcription unit was reassembled using standard techniques, deleting the sequence between the introduced sites. Construction of RNase probes specific for the $\Delta 4\text{Ac}$ and $\Delta 5\text{Ac}$ transcription units has been described in Fig. 1. A 550-bp fragment of the $\Delta 4\text{Ac}$ transcription unit extending from a position 91 bp upstream from the fourth exon to the end of the calcitonin-specific exon was fused to sequences corresponding to 131 bp from the 3' end of the fourth intron and the complete CGRP-specific exon (exon 5). An RNase probe specific for the $\Delta 5\text{Ac}$ transcription unit was constructed containing a 154-bp region from the 3' end of the third intron and the entire calcitonin-specific exon (exon 4) fused to a 251-bp DNA fragment containing 165 bp of exon 5 sequence information and 85 bp of adjacent intervening sequence information from the fourth intron.

spliced products. To examine putative *cis*-active elements involved in alternative splice-site selection, initial attention focused on intervening sequence information between the third and fifth exons. Intron deletions were designed to conserve *cis*-active sequences necessary for constitutive splicing, including the 5'-donor, lariat branchpoint, 3'-acceptor and polyadenylation signal^{3,5,14,15}. A 380-base-pair (bp) fragment beginning 77 bp downstream from the exon-3 splice donor site was deleted from the third intron of the calcitonin/CGRP gene (Fig. 2a). Ribonuclease protection analyses of HeLa cells expressing this transcription unit (Δ Int3) revealed that calcitonin mRNA continued to be the predominant product, representing 98% of the mature RNA species, whereas F9 cells still produced >97% CGRP transcripts (Fig. 2b). Analyses of a 590-bp deletion in the fourth intron beginning 55 bp downstream from the end of the fourth exon (Δ Int4; Fig. 2a) revealed that deletion of most of the fourth intron also had little effect on alternative RNA processing in these cell lines (Fig. 2b). These results demonstrated that most of the RNA sequence information within the third or fourth introns was not essential for alternative splice-site selection.

CGRP mRNA production could reflect the actions of either a positive *trans*-acting factor(s) that promotes use of the CGRP-

specific splice acceptor or a negative factor that inhibits the use of the calcitonin-specific splice acceptor. Previous studies have suggested that removal of a 369-bp fragment from the 3' end of the third intron results in the production of significant levels of CGRP mRNA in a calcitonin-producing cell line¹¹. To examine the role of sequence information at the calcitonin-specific acceptor itself, a 77-bp fragment containing the exon 4 3'-splice junction was deleted from the calcitonin/CGRP transcription unit (Δ 4Ac; Fig. 3a). The 5' end of this deletion was chosen to minimize any cryptic splice acceptor selection by removing a potential branchpoint sequence located 55 nucleotides upstream from the 5' end of the fourth exon¹⁶. Transfection of this transcription unit into HeLa cells resulted in the production of mature CGRP mRNA, yielding an RNA-processing pattern virtually identical to F9 cells transfected with the wild-type gene (Fig. 4b). Because calcitonin-producing cells could use the CGRP-specific splice acceptor, there appeared to be no mandatory requirement for a positive cell-specific *trans*-acting factor(s) to promote exon 3 to exon 5 splicing for generation of mature CGRP mRNA.

To investigate the potential role of an inhibitory factor acting at the calcitonin-specific 3'-splice junction, the ability of CGRP-producing cells to alter their splice-site selection in favour of calcitonin mRNA production was investigated by removal of the CGRP-specific splice acceptor site (Δ 5Ac; Fig. 3a). The 5' end of this 206-bp deletion removed a potential branchpoint sequence, just upstream of a 150-nucleotide polypyrimidine stretch before the CGRP-specific splice acceptor site, and continued 18 nucleotides into the fifth exon¹⁷. Transfection of this mutant transcription unit into HeLa cells resulted in the normal production of mature calcitonin mRNA; however, F9 cells produced only unprocessed RNA species (Fig. 3b). These results suggested that, in the absence of a functional CGRP-specific splice acceptor, CGRP-producing cells were incapable of altering their differential splice-site selection to produce calcitonin mRNA, consistent with a cell-specific inhibition of the calcitonin splice acceptor site.

To further examine *cis*-active elements near the calcitonin-specific splice acceptor site, a series of calcitonin/CGRP transcription units were constructed containing nested deletions

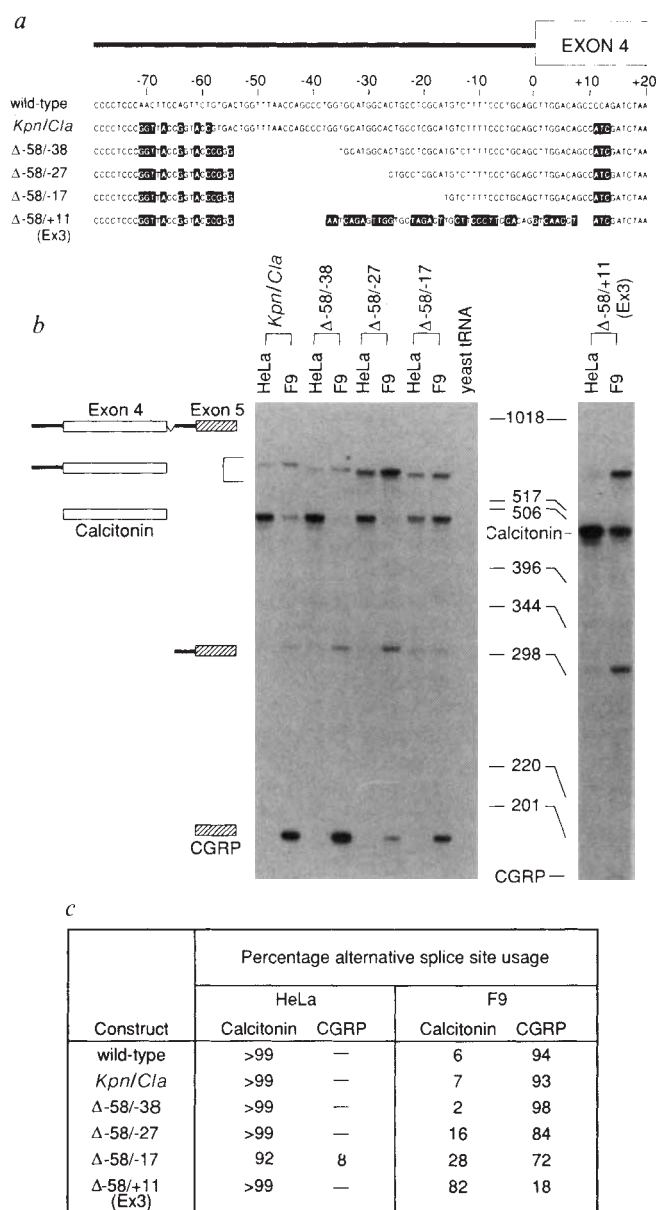


FIG. 4 Deletion or replacement of sequence information at the calcitonin-specific splice acceptor alters the RNA-processing choice of F9 cells. **a**, Nucleotide sequences of wild-type and mutant calcitonin-specific splice acceptors. Alterations from the wild-type sequence are indicated by the boxed letters. **b**, Ribonuclease protection analyses of RNA from permanently transfected HeLa and F9 polyclonal cell lines expressing calcitonin/CGRP transcription units containing mutations of the calcitonin-specific splice acceptor region. A progressive decrease in the size of the protected RNA fragment corresponding to an RNA species in which splicing to the calcitonin-specific acceptor has not occurred (bracketed), results from the progressively larger deletions. The structures and migration positions of predicted RNase protection fragments and radiolabelled DNA markers are indicated; yeast transfer RNA (20 μ g) was included as a negative control. **c**, Densitometric quantitation of the relative levels of calcitonin- and CGRP-specific splice acceptor use in permanently transfected HeLa and F9 polyclonal cell lines. **METHODS**. Mutations of the calcitonin-specific splice acceptor were made by introduction of unique *Kpn* I (GGTACC) and *Cla* I (ATCGAT) restriction sites as indicated in panel **a** by oligonucleotide-directed mutagenesis in M13mp18 (ref. 28). Mutations were generated by insertion of double-stranded synthetic oligonucleotides into the introduced sites in the calcitonin/CGRP transcription unit; an internal *Sma* I restriction site (CCCGGG) was included in each insert for diagnostic purposes. Ribonuclease protection and densitometric analyses were performed as described in Fig. 1. RNase protection probes specific for each transcription unit were generated as described in Fig. 1. Each probe contains sequences from the 3' end of the third intron, from a common endpoint 154 bp upstream from the fourth exon in the wild-type gene, to the end of the calcitonin-specific exon (exon 4) fused to sequences corresponding to 131 bp from the 3' end of the fourth intron and the complete CGRP-specific exon (exon 5).

from a common 5'-endpoint 58 nucleotides upstream from the fourth exon (Fig. 4a). Introduction of *KpnI* and *ClaI* restriction sites alone, allowing construction of these nested deletions, had little effect on cell-specific alternative splice-site selection (Fig. 4b, c). Deletion of a region between -58 and -38 (Δ -58/-38) had no apparent effect on alternative splicing. Further deletion from -58 to -27 (Δ -58/-27) resulted in continued use of the calcitonin acceptor in HeLa cells with increased levels of unprocessed RNA precursors. F9 cells transfected with this mutant transcription unit produced increased levels of unprocessed RNA species and an apparent increase in the ratio of calcitonin to CGRP mRNA. Additional deletion from -58 to -17 (Δ -58/-17) further increased the percentage of calcitonin-specific splice acceptor use in the CGRP-producing cell line. Sequence analysis of RNA from this transcription unit confirmed that accurate splicing of the exon 3 donor to the exon 4 acceptor had occurred in both cell types (data not shown). These results suggested that a *cis*-active element near the calcitonin acceptor inhibited the formation of calcitonin transcripts in F9 cells and that deletion of this element(s) permitted the increased production of calcitonin mRNA in the CGRP-producing cell line.

Further nested deletions near the calcitonin-specific acceptor were not feasible owing to the presence of the splice acceptor consensus sequence, (Py)₁₀GCAG, which has previously been shown to be critical for constitutive splicing¹⁸. In order to further define *cis*-active elements at the calcitonin acceptor itself, the entire splice acceptor region, corresponding to the coordinates -58 to +11 was removed and replaced with a heterologous splice acceptor that could be used by both cell types (Δ -58/+11 (Ex3); Fig. 4a). We chose a region of the exon 3 splice acceptor from -39 to +8 relative to the beginning of the third exon. When transfected into HeLa cells, this transcription unit resulted in the production of calcitonin transcripts only, whereas F9 cells now produced ~82% calcitonin mRNA (Fig. 4b, c). Sequence analysis of RNA from F9 cells transfected with this transcription unit confirmed the accuracy of splicing (data not shown). These results demonstrated that removal of sequence information from the calcitonin-specific 3'-splice junction and its replacement with an acceptor that could be used by F9 cells resulted in the efficient production of mature calcitonin mRNA in a CGRP-producing cell line.

We have demonstrated that tissue-specific processing of the calcitonin/CGRP primary transcript is the result of alternative splice-site selection primarily regulated by *cis*-active sequences at the calcitonin-specific 3'-splice junction. Deletion of sequence information at the calcitonin acceptor results in an alteration of splice-site selection in CGRP, rather than calcitonin-producing cells. These results are most consistent with a model in which *cis*-active sequences serve to inhibit the formation of calcitonin mRNA in the CGRP-producing cell line. Recent genetic analyses of developmental loci in *Drosophila melanogaster* have suggested that specific gene products, with putative RNA-binding domains, may regulate alternative splicing in the sex-determination pathway¹⁹⁻²¹. Such *trans*-acting factors may affect differential RNA processing by binding to specific *cis*-active pre-mRNA sequences, thereby promoting or repressing the selection of alternative splice sites²²⁻²⁴. Further definition of the *cis*-active element(s) involved in the production of calcitonin and CGRP messenger RNA may help to isolate and characterize the cell-specific *trans*-acting factor(s) involved in the tissue-specific RNA processing of this gene. □

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CORRECTION

Spatial and temporal expression of the retinoic acid receptor in the regenerating amphibian limb

Vincent Giguère, Estelita S. Ong, Ronald M. Evans & Clifford J. Tabin

Nature **337**, 566-569 (1989).

As part of the above report of the temporal and spatial expression of a retinoic acid receptor (RAR) during amphibian limb regeneration, we isolated cDNA sequences from a regenerating newt cDNA library using a human RAR α probe (in humans two distinct RARs were known, called RAR α and RAR β). As described, two non-overlapping cDNAs were interpreted as representing contiguous portions of a single newt gene product. A chimera constructed by fusing the two clones at a common *EcoRI* site was shown to function as a retinoic acid receptor in an *in vitro* assay.

Recent work by C. Ragsdale, P. B. Gates and J. P. Brockes has resulted in the isolation of a newt RAR cDNA referred to as RAR δ whose 3' sequence is identical to clone NB6 in our report but whose 5' sequence differs from one clone NB8. This suggests that the two cDNAs we isolated originated as transcripts from two distinct, although closely related genes. With 5' sequence information provided by J. Brockes and colleagues, we have used the polymerase chain reaction (PCR) to examine both genomic DNA and newt mRNA. The results verify the existence of two distinct genes and gene products. Moreover, RNase protection experiments using a probe spanning our chimeraic junction confirm that the two sequences we fused are not found on a contiguous RNA transcript. Accordingly, our published sequence should be interpreted as representing the partial sequence of two different genes: RAR β (the clone NB8 in our paper) and a novel β -like RAR (clone NB6).

Since the probe used for our *in situ* hybridizations was derived solely from the clone NB8 the revised sequence interpretation does not affect the data or the conclusions presented in our paper. In addition, our work together with the results from the Brockes laboratory leads to the intriguing conclusion that there are at least two β -like RARs expressed in the regenerating limb. □

Environmental SEM premières

N. Baumgarten

The environmental scanning electron microscope (SEM) eliminates the high-vacuum requirement of conventional SEM, allowing the analysis of unprepared, wet samples.

DURING the past three decades, the scanning electron microscope (SEM) has opened a window into the fascinating, often beautiful, 3-dimensional microcosm beyond the reach of glass optics. In principle, the SEM works like a television cathode-ray tube, with an electron gun, deflection coils and focusing lenses. Its beam path, or column, must be kept scrupulously clean and evacuated to pressures near those found on the surface of the moon. A fine flow of electrons, focused to a point less than one micron in diameter, is scanned over the specimen surface in a raster pattern, while the 'reflected' electron signal modulates the brightness of a synchronously scanned display screen. Image magnifications from $10\times$ to $100,000\times$ and beyond may be obtained by varying the size of the projected raster.

From the beginning, however, there have been large classes of objects whose physical properties kept them 'off-limits' to electron beam inspection by making them incompatible with the SEM's high-vacuum environment. The near-impossibility of viewing wet, oily or electrically insulating specimens in their natural states has given rise to the routine use of preparation techniques such as drying, freezing, fracturing and conductive coating¹. But even with specimens that can be prepared to be tolerated by the SEM's vacuum system, there is no simple way to determine whether the structures of interest have been altered or masked by the preparation process². Certain kinds of materials, especially in biological fields, have until now effectively resisted all attempts at direct SEM imaging.

Although sporadic attempts have been made over the years to render the SEM accessible to real-world specimens^{3,4}, none has ever gained wide acceptance because of the various complications and sacrifices in performance required. Only recently has an instrument been developed that can make high-resolution secondary-electron images of virtually any specimen, regardless of composition. The environmental SEM (or E-SEM) (Fig. 1) is the result of ten years of experimental work by Australian researcher G. D. Danilatos, followed by a sizable engineering effort financed by a group of US venture capital firms.

Originally conceived as a fairly specialized piece of equipment, the E-SEM has demonstrated its near-universal applicability, combining the high resolution and

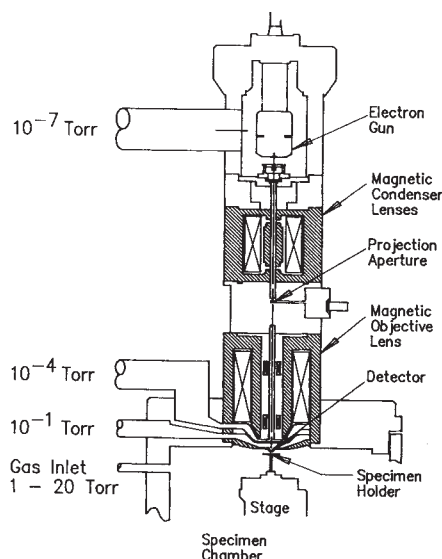


Fig. 1 The optical column and differential pumping system of the environmental scanning electron microscope.

field depth of the SEM with the flexibility and ease of the light microscope. This long-sought goal has now been achieved through a blending of existing technologies and the serendipitous discovery of a new signal detection method.

Design hurdles

Designing a scanning-beam instrument to accept any and all specimens presents a number of practical problems. Foremost among these is the necessity to avoid contaminating the clean, high vacuum required in the gun chamber with the moist, high-volatility atmosphere of the specimen chamber. The problem is overcome in the E-SEM through the use of a standard vacuum technique known as differential pumping, in which a series of chambers at increasing pressures along the beam path are linked by small apertures. Dedicated pumps at each stage maintain the required pressure gradient and stop contaminants from reaching the clean upper column. The specimen environment can then be held at pressures exceeding 20 torr (26 mbar), the saturated partial pressure of water vapour at room temperature. At these pressures, wet specimens remain fully hydrated during examination, and liquids may even be observed.

Early studies of electron beam scattering in air led to the assumption that there was little possibility of being able to project a beam fine enough for microscopy through a gas of any significant

density. Later re-examination of the problem showed that only a fraction of the beam electrons are scattered into a diffuse 'skirt' if the path length through the high-pressure region is kept short enough, with the remainder of the electrons being left in their original focused trajectories. The surprising result of this finding is that the unscattered beam fraction can still be used to form an image with the same resolution as that obtained in a high vacuum⁵. (The loss in signal-to-noise ratio can generally be made up in other ways.) To make practical use of this result in the E-SEM, it was necessary to place several stages of the differential pumping system inside the final magnetic lens.

Detector development

The development of a secondary electron detector able to operate at elevated chamber pressures was also critical to the success of the E-SEM as a general-purpose imaging tool, because low-energy secondary electrons from the beam impact point are the carriers of the highest-resolution imaging signal⁶. (The usual Everhart-Thornley detector of the high-vacuum SEM cannot be used at these pressures because of unwanted gas ionization and breakdown caused by the high voltage on its scintillator surface.) The E-SEM uses a variation of the gaseous detector device first described by Danilatos in 1982, which involves the gas itself in a signal amplification process^{7,8}. Now called the environmental secondary detector (ESD), this simple, compact system is in turn physically integrated into the electron optics and vacuum system.

The new detector takes advantage of the well-understood ionization behaviour of the low-pressure gases found in the E-SEM specimen environment. Gas ionization is induced by a moderate electric field, forcing collisions between highly mobile secondary electrons and neutral gas molecules, resulting in the cascading multiplication of the secondary signal. The slow-moving positive ions present in the gaseous environment also effectively neutralize troublesome surface charging of insulating specimens, regardless of the beam acceleration voltage. Coaxially mounted within the final lens, the detector has perfect symmetry, excellent collection efficiency, and will function with almost any gas.

E-SEM inspection of specially prepared thin-film specimens has shown that at typical operating pressures, the ESD

signal is produced almost exclusively from surface secondary electrons, instead of the higher-energy backscatter electrons which are a large component of the SEM secondary signal. Unlike a SEM detector, the ESD is sensitive to the energy spectrum of secondary electrons, information which is at present lost in a high-vacuum SEM. The ESD is also insensitive to light, allowing red-hot or otherwise light-emitting specimens to be observed easily.

Initial applications of the E-SEM have included dynamic studies of crystal growth, absorption, drying, melting, corrosion and other chemical reactions. The instrument is suitable for the direct observation of paper, plastics, ceramics, textiles, foods, fibres, pharmaceuticals, ores, semiconductors, and many other difficult SEM specimens, including biological samples. □

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Improving your image

A troubleshooting modem link for EM hardware and a high-sensitivity imaging plate for TEMs are a few of the new ideas for microscopy that can be seen at EMAG-MICRO '89 in London next week.

POLAROID (UK) Ltd says its Presentation Copier can at the touch of a few buttons produce full-colour or monochrome slides, prints and overhead transparencies from existing micrographs, documents or artwork (*Reader Service No. 101*). The £3,500 (UK) presentation copier, which takes up no more space than a standard desktop copier, has a control panel that can be operated by users with no photographic experience. The system incorporates an auto-winding motorized 35 mm camera back, a pack-film camera back, and preprogrammed settings for five different slide films, colour prints and mini-overheads. The presentation copier accepts all Polaroid instant 35-mm slide films for producing full-colour, black and white, or white-on-blue slides, and two of Polaroid's 3.25 x 4.25-in. instant peel-apart films for full-colour prints and mini-overheads. Polaroid will be exhibiting on stand 46.

Cameca UK Ltd, on stand 2, will be featuring a **telephone modem** that enables SEM hardware to be operated from a remote computer workstation (*Reader Service No. 102*). The company will be demonstrating a computer workstation operating a Cameca SX50 electron microprobe analyser situated in the company's Applications Laboratory in Paris. The modem link facilitates the exchange of data between users and allows remote interrogation of the machine's electronics.

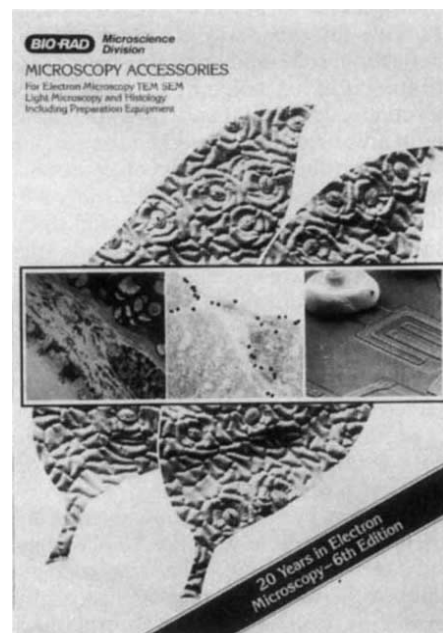


Cameca Ltd's modem link for remote SEM operation.

Apart from the obvious advantages of image data exchange between users, the modem should allow remote fault diagnosis by a field engineer located in the company's support centre, without the need, in many cases, for direct engineer intervention.

To celebrate 20 years in electron microscopy, Bio-Rad Microscience Division,

exhibiting on stand 29, announces its 165-page **catalogue of microscopy accessories** (*Reader Service No. 103*). Divided into 33 sections, the catalogue covers all aspects of microscopy, with specific sections on



Bio-Rad's 20th anniversary edition microscopy catalogue.

SEM and TEM preparation, histology, asbestos sample preparation and immunogold labelling. Other more general sections cover processing chemicals, embedding, ultramicrotomy, metallurgy, calibration aids and preparation equipment.

Analytical Measuring Systems, in collaboration with Microtest Research Ltd, has produced an image analysis system that automates the counting process involved in **Ames bacterial mutagenicity testing** and inhibition zone analysis (*Reader Service No. 104*). The system includes a model 40-10 image analyser, macrostand and lens kit, IBM PC or compatible computer, and plate count analyser software. The system can be used for automated high-speed counting of bacterial colonies on nutrient plates. The software enables the user to carry out a series of statistical analyses on experimental count data, formulate data into tables, calculate mutagenicity increases over controls, display and print graphs and raw data tables, and edit reports. AMS says the system is designed to enable users to re-format reports to suit their own experimental and reporting protocols. Analyti-



Automated colony counter and analyser for Ames testing from AMS.

cal Measuring Systems will be exhibiting on stand 23.

Sample preparation

Balzers High Vacuum Ltd says its new model CPD 030 **critical point dryer**, which will be exhibited on stand 44, is ideal for drying delicate botanical, zoological or industrial specimens before examination by SEM (*Reader Service No. 105*). Specimens are loaded into the pressure chamber, which is cooled to a preselected temperature of between 2 °C and 12 °C. Large observation windows at the top and side of the specimen chamber allow the user to view the drying process. Liquid CO₂ enters the chamber, is stirred and then partially drained out several times until the specimen is immersed in pure CO₂. The chamber temperature is then increased to between 30 °C and 45 °C, which causes the pressure to rise; at the "critical point" the liquid medium changes



Balzers Ltd's critical point dryer for delicate specimens.

to the gaseous phase and is vented from the chamber. Safety features on the £2,275 (UK) model CPD 030 include a pressure container approved to 200-bar pressure with a bursting membrane at 150-bar pressure.

The model CT 1500 Cryotrans preparation system for **cryoscanning electron microscopy** will be exhibited by Oxford Instruments Ltd on stand 43 (*Reader Service No. 106*). The modular machine comes in two models: the CT 1500B standard system with facilities for gold sputter-coating and carbon evaporation, and the CT 1500C system with a full range

of accessories and options. The system uses rapid freezing techniques, which the company says allows specimens to remain fully hydrated with their internal structures preserved. Oxford Instruments says samples can be prepared and viewed in minutes, eliminating the need for time-consuming chemical fixation methods and solvent immersion. Fracturing, sputter-coating, carbon and metal evaporation are carried out in a compact preparation chamber equipped with a nitrogen cooling stage. Oxford says temperature regulation is accurate to ± 0.5 °C and one filling of the liquid nitrogen dewar lasts four hours.

Digitally-controlled operation and programmable sample preparation are features of the Multipol 3 — Malvern Instruments Ltd's three-station **polishing machine** (*Reader Service No. 107*). The company says the Multipol 3 is suitable for



Multipol-3 — the multipurpose polishing machine from Malvern Instruments.

all lapping and polishing methods, including abrasive slurry polishing, and diamond and chemical etches. The machine polishes samples to high degrees of flatness and parallelism, with excellent sample-to-sample consistency, says the company. An extensive range of polishing plates are available: the 300 mm-diameter lapping and polishing plate can handle samples of up to 125 mm in diameter. Over 200 different lapping and polishing protocols can be stored in the system memory, which allows the user to create new protocols or modify existing ones. The Multipol 3 will be on display on stand 45B.

Testbourne Ltd says its Hummer VII **sputter coater** can coat SEM specimens with uniform films with grain sizes of less than 2 nm (*Reader Service No. 108*). The microprocessor-controlled Hummer VII can operate both automatically or manually and can coat specimens of up to 3.25 inches in diameter. When in automatic mode, the user selects the desired film thickness, which can be from 1 to 999 nm, and sets the other operating parameters — no further adjustments of voltage, current or vacuum are necessary. The planar magnetron sputter deposition source produces a cool plasma and can sputter-



The Hummer VII automated sputter coater from Testbourne Ltd.

deposit most precious metal targets, including gold and gold/palladium. The chamber is automatically vented after processing to prevent particulate turbulence. The system offers a pulse deposition mode for fine-grain thin film nucleation, and an etch mode for the surface-cleaning of contaminated specimens prior to sputter coating. The company will be exhibiting on stand 32.

Conjugates

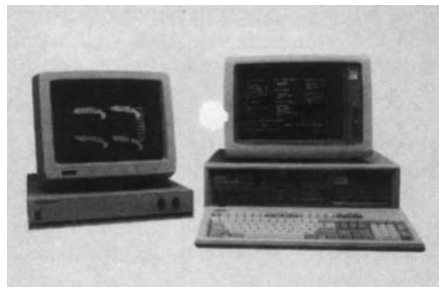
BioCell Research Laboratories has added **1-nm gold conjugates** of goat anti-rabbit IgG and goat anti-mouse IgG to its range of immunogold reagents (*Reader Service No. 109*). The conjugates are used in combination with BioCell's new silver enhancing kits, which amplify the gold signal for antigen detection by light microscopy or immunoblotting. The company says the new conjugates allow increased penetration of cells and tissues without the need for severe permeabilizing procedures. Because the 1-nm gold particles are smaller than immunoglobulins, each can carry several particles with minimal effect on antibody conformity, says BioCell. The silver enhancing kits are insensitive to light and can be stored at 4 °C for at least six months. The gold conjugates are stable for 12 months at 4 °C, and can be stored almost indefinitely when frozen. BioCell will be on stand 28.

Vector Laboratories, exhibiting on stand 8, has a host of **fluorescent and enzymatic reagents** for use in immunohistochemistry, EM, in-situ hybridization, transfer blots and flow cytometry (*Reader Service No. 110*). Fluorescently-labelled streptavidin is available conjugated to fluorescein, Texas Red, phycoerythrin or AMCA — a new blue fluorescent dye. Enzyme-conjugated streptavidin is available labelled with either horseradish peroxidase or alkaline phosphatase. Vector says its streptavidin conjugates are ultrapure and give a high signal-to-noise ratio. For colloidal gold labelling, Vector offers ultrapure streptavidin in an unconjugated form. A selection of affinity-purified anti-biotin and anti-avidin D antibodies in labelled and unconjugated forms are also available.

Fluorescence microscopy

In response to the current popularity of non-disruptive methods for measuring intracellular ion concentration using fluorescent dyes, Nikon UK Ltd now offers a **microspectrofluorimetry system**, which will be on show on stand 24 (*Reader Service No. 111*). The system — developed at Newcastle University — is based on Nikon's Diaphot epifluorescence microscope. It makes use of the Diaphot's short path length and a Nikon beam splitter. When configured for use with a dichroic mirror, the system allows the simultaneous measurement of light emitted at two wavelengths by fluorescent dyes specific for calcium, hydrogen and sodium ions. The machine features a twin-camera port, which allows output from the system to be fed simultaneously to an image analyser and a photon counter. The £20,000 (UK) system can be used to detect intracellular ionic levels and monitor concentration changes across the cell and over time.

Hamamatsu Photonics has an **image processing system** designed specifically for dual-wavelength fluorescence microscopy that can analyse and calculate changes in intracellular ion concentration (*Reader Service No. 112*). The ICMS system includes a low-light level video camera, real-



The Hamamatsu image processing system for dual wavelength fluorescence microscopy. time image processor and IBM/AT PC or compatible computer. Functions performed by the PC include filter changing, image acquisition and analysis, scaling, and the graphic display of results. The system can be used for screening cells before using the zoom function for more detailed analysis. Other uses include studying intercellular communication and creating 2-D displays of a cell to demonstrate local changes in calcium, hydrogen, potassium, and sodium ion concentrations. Hamamatsu says the system's time resolution of three to four pairs of images per second, with a size of 256×340 pixels, is more than adequate for many biological applications. The company will be exhibiting on stand 53.

Electron microscopy

To overcome the disadvantages of photographic film as an image-recording material in TEM, Jeol Ltd and Fuji Photo Film Company Ltd have developed an **imaging plate** made of a highly sensitive X-

ray recording material (*Reader Service No. 113*). Jeol says the plate is 1,000 times more sensitive than photographic film, and yields a linear response over the full range of electron exposure. The imaging plate is part of the PIXsysTEM, which also includes a TEM with an imaging plate camera, an imaging plate reader/eraser, processor, and printer. Jeol says the imaging plate produces a TEM digital image measuring 102×77 mm, with 2048×1536 pixels and 4096 grey levels. The final image can be stored on optical disc or

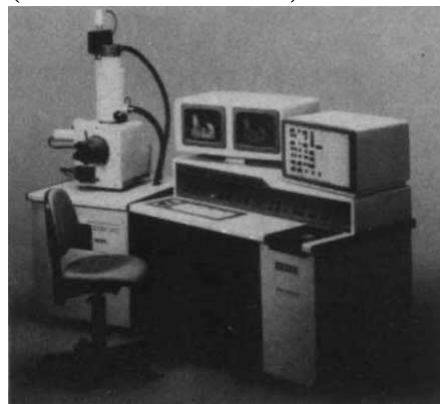


Contour map from Jeol's PIXsysTEM.

printed. After processing, the imaging plate can be erased and reused — eliminating the need for dark-room processing. Jeol will be on stand 18.

Link Analytical Ltd will be showing off its new **EM data management system** — the model eXL (*Reader Service No. 114*). The system features a Pentafet X-ray detector, computer-controlled pulse processor and digital imaging software, which Link says offers powerful electron image acquisition and fast data processing. An extensive range of software is available for quantitative X-ray microanalysis, image analysis, data processing, and full EM automation. The model eXL analytical workstation can be seen in operation linked up to a Zeiss DSM 960 EM, on stand 20.

Science-fiction enthusiasts who have seen "Star Trek V" may be interested to know that Carl Zeiss, Inc.'s model DSM 960 **scanning electron microscope** was used to create some of the film's special effects (*Reader Service No. 115*). The model



The DSM 960 from Carl Zeiss.

DSM 960 features a spacious specimen chamber to which detectors for SE, BSE, CL, EDX and WDX spectrometers can be fitted. The digital SEM has microprocessor-control over all operations, including output, stage movements and microscopic functions. A digital frame-store allows the operator to view on the monitor what the SEM is seeing, with no "noise" when on slow scan, says the company, and without the need to first capture the image on film. Zeiss will be demonstrating the model DSM 960, which sells for about £78,000 (UK), on stand 4.

Hitachi Scientific Instruments is launching its model S-2700 computerized **scanning electron microscope**, which can be used in a normally lit room (*Reader Service No. 116*). The model S-2700 features Hitachi's conical objective lens, which the company says allows energy-dispersive X-ray analysis and wavelength-dispersive X-ray analysis to be performed at high take-off angles (35 per cent) with improved collection efficiency at a short working distance (12 mm). High-resolution SEM and backscatter photographs can be taken by non-specialists, says Hitachi, because there is no need to change the Z position for X-ray analysis, even for large or highly tilted samples. The SEM is equipped with two built-in frame stores — one of which may be colour — which allow flicker-free TV-scan images to be viewed on the monitor, even when the beam is not on the sample, says Hitachi. The S-2700 can be interfaced with an image analysis system, and can be seen on stand 37.

The model CM20 **scanning transmission electron microscope** from Philips Scientific is based on the optics of Philips' model CM12 and has full 200 kV-power (*Reader Service No. 117*). The system features Philips' patented TWIN lens, which the company says gives high performance in both TEM and probe-forming modes, and a eucentrically tilting goniometer stage and wide range of holders. The CM microprocessor-based control system linking the operator console with the column hardware allows software to be matched to the applications requirements of the user. Philips says the complete CM20 system provides high-resolution in imaging and microanalytical techniques, with facilities for STEM and SEM operation. The model CM20 can be interfaced with TV cameras, X-ray micro-analytical systems and electron spectrometers.

Microscopes and more

Visitors to stand 55 will be able to see W.A. Technology's **scanning tunnelling microscope** (*Reader Service No. 118*). The STM can image and analyse spectroscopically surfaces on an atomic scale — in air, liquid or vacuum. The system is comprised of a scanning head, electronic con-

trol unit, IBM AT 286/386 or compatible computer with a colour monitor, monochrome frame store and monitor, and dedicated software. The fine metal tip — whose position is controlled by piezo-electric elements — is placed within a few atom diameters of the specimen surface. A voltage applied between the tip and specimen produces a tunnelling current. As the tip scans the surface, its height is adjusted to keep the current constant, and the Z-control voltage provides a readout of the surface profile. Menu-driven software controls most STM functions in both topographic and spectroscopic modes, including parameter setting, running scans or data point samples, and image and data processing.

Wild Leitz will be taking the opportunity to show off its **confocal laser scanning microscope** for 3-D image processing, on stand 3 (*Reader Service No. 119*). The CLSM is based on Leitz's Diaplan microscope, and uses the latest confocal laser technology in combination with a high-resolution image processing system. Leitz says the CLSM is optimized for scanning biological tissues and cell structures, particularly structures with minimum contrast. The CLSM optimizes fluorescent



The Wild Leitz confocal laser scanning microscope.

yield by eliminating stray light and minimizing sample bleaching due to its pulse laser operations, says the company. The microscope can function in reflection and transmission mode, and can be fitted with two channel detectors. The package is rounded off with a wide range of software for 2-D and 3-D image processing, including image filtering, image combinations, densitometry, extended focus, height and contour measurements, 3-D plots, stereo imaging, pseudo-colour, results processing and statistical management.

Olympus Optical will be exhibiting a complete new range of **zoom stereo-microscopes** for use in fields as diverse as biomedicine to semiconductor research, on stand 38 (*Reader Service No. 120*). Key features of the microscopes include newly designed Greenough-type optics, which the company says provide excellent depth of field and image flatness with a large working distance. The company will also be introducing two new student microscopes in the CH series: the CHK and CHL, both of which are equipped with a sturdy metal stand and heavy-duty stage,

in order to withstand the rigours of classroom use.

Image processing

Colour Oasis — a full 24-bit **colour archiving system** — will be on display in Sight Systems' stand, number 26 (*Reader Service No. 121*). The £8,000–15,000 (UK) system captures frames of colour video information in analogue format from VTRs, optical and electron microscopes, and video cameras, and converts the information into a high-resolution digital image which is stored on a removable optical disc. Sight Systems says Colour Oasis offers fast archiving and retrieval of up to 880 full-colour images per disc. The system incorporates a new optical disc drive which has a 940-Mbyte capacity. Images are stored in tagged image file format, which the company says is compatible with desktop publishing programs. The system enables users to import high-resolution video images into reports and documents produced by word-processing.

sysTEM — Synoptics' new **image processing and enhancement** system for TEM hardware — will be in action on stand 51 (*Reader Service No. 122*). Designed for operation by non-specialists, sysTEM captures images directly at video rate from a TV camera attached to the TEM. Besides noise reduction, sysTEM produces near-real-time calculations which allow the user to focus and align the TEM accurately. The basic system includes a monitor with a mouse-controlled pointer, the sysTEM unit, and host computer. The computer directs sysTEM in its video rate processing and display operations, provides disc/tape storage of images, executes the Semper 6 plus software program, and can be networked to workstations — each of which can access image archives acquired by sysTEM for off-line processing. Direct on-line image comparisons can be made on sysTEM's screen by importing simulated images from the workstation or host computer.

Cambridge Instruments Ltd, exhibiting on stand 1, announces the Quantimet 570 — an image analysis system for true **colour image processing** and analysis (*Reader*



Cambridge Instrument's Quantimet 570 image analyser.

Service No. 123). The company says the programmable image array processor and software is optimized and based on the principles of mathematical morphology, developed in conjunction with the Centre de Morphologie Mathematique in Paris. Stated applications include the analysis of stained tissue sections and polished mineral sections when lit with polarized light. The Quantimet 570 allows the user to distinguish a full range of hues as well as neutral tones. Pseudo-colour modes are available, and the system has 64 full-resolution image frames. Other features include image averaging and integration to reduce "noise", and the capacity for zooming, shrinking and rotating.

Quantel Ltd's Sapphire image processing system now includes software for **measurement and densitometry options** (*Reader Service No. 124*). Both packages offer a range of quantitative and analysis facilities implemented by defining a line or box on the screen for point-to-point or area analysis of luminance distribution; information can be displayed either graphically or numerically. The £1,500 (UK) measurement option provides on-line calibrated measurements directly from the image. Depending upon whether a line or area is defined, operating modes include direct or graphic averaged line or column measurements. Using these basic processes, automated measurements can be performed. The £1,885 (UK) densitometry option is now offered as a result of Quantel's development of real-time shading correction. Quantel will be on stand 35. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

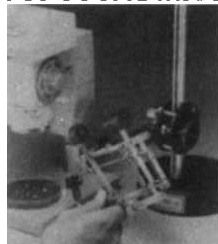
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When will biotechnology mature?

Richard Pearson

The science of 'biotechnology' will be judged during the 1990s by what new products are launched, not by promises. But there will still be a demand for qualified workers.

BIOTECHNOLOGY is now entering a critical phase as the multinational chemical and pharmaceutical companies move closer to the centre of action via a mixture of indigenous growth and selective takeover. In its early days, biotechnology was driven by the push of the scientists and the only constraints were those of feasibility. Now the needs of the marketplace and cost control are paramount.

Progress so far has been rapid, and is expected to continue to be so in pharmaceutical, agriculture and food, but lower oil prices are expected to slow developments in chemicals and the environmental opportunities are still likely to take some years to realize.

Overall the OECD (Organization for Economic Cooperation and Development) sees that the short-term impact on employment is likely to be negative due to rationalizations and cost reductions, with cause and effects often appearing in different continents as Third-World producers for example lose out as crop yields increase. What is seen as certain is the continually rising qualification profile of those working in the industry and the need for the continual updating of skills and retraining¹.

In the United Kingdom, one-third of the new recruits last year to biotechnology work were graduates, while 17 per cent had PhDs and a further 20 per cent had postdoctoral qualifications. Half of these recruits entered work related to bioprocess technology.

The perceived value of doctoral programmes in biotechnology is shown by the way the level of student interest has held up throughout the 1980s despite improving job attractions for first-degree graduates and the falling value of postgraduate grants. Most doctoral students pursued their postgraduate study because of the intrinsic interest of the subject and found places on their own initiative or with the help of their undergraduate supervisor; few were attracted by advertising or the work of the careers services. In contrast, those going on to masters' courses were more likely to see them as an opportunity to gain access to the biotechnology labour market.

Funding

In 1988 there were over 1,100 full-time PhD studentships in biotechnology and around 200 at masters' level. At PhD

level, the dominant funding source was the SERC (Science and Engineering Research Council), which accounted for about 45 per cent of the studentships awarded, although only a minority of these were funded through the specialist Biotechnology Directorate, the others being funded through the more general science boards. Non-SERC sources included the other research councils (accounting for about 10 per cent), industry, academic institutions, private and other sources; overseas funds, including those directed through the British Council, accounted for nearly one in five students. This proportion of research-council funding in biotechnology is rather higher than the 35 per cent across all the sciences.

At masters' level, the main funding sources were private funding (one third) while the Training Agency (formerly the Manpower Services Commission) and the British Council each funded approximately 10 per cent of the total. SERC funding was low because it concentrates on support at doctoral level in answer to the needs of industry.

A survey of postgraduates completing their research since 1982 showed that one in three were women, their representation being highest on masters' courses, and that the mean age of all the students was 26. While fewer than 10 per cent of those taking part in PhD programmes had degree classes below an upper second, one in five of those on masters' courses had such degrees. Interestingly a higher proportion of students from sandwich courses were to be found on the PhDs' than on the masters' courses. In terms of source disciplines, biochemistry and microbiology dominated, followed by applied biology and chemistry and chemical engineering.

Once into their postgraduate study, most students found their needs for interest being met, and felt they received a good standard of supervision and provision of training in research techniques. But concern was expressed about the lack of training in team working and there were complaints at the low value of maintenance grants and awards.

The possession of a PhD was seen as an advantage both in getting a job on completion of the course (80 per cent entering biotechnology related employment) and in subsequent performance in it. A masters' degree was seen to be rather less

valuable and reflected lack of relevant experience and the weaker demand in the labour market for this qualification. Only 66 per cent of those with masters' degrees entering biotechnology-related employment.

Employers reported that skill shortage remained selective and that while the supply of PhDs was growing, the quality seemed to be falling. The range of shortages now also embraces specialists in plant molecular biology, cultures, microbial physiologists and downstream processing. The problems are worst in higher education, where the ability to offer jobs only on short-term contracts and inflexible salary structure are major barriers to recruitment².

Management

In addition to a continuing supply of doctoral and postdoctoral graduates, training needs in the future are seen to centre on scientific updating where distance learning will become increasingly important. Following the recent expansion in the supply of short courses, of which more than 75 now exist, provision is thought to be saturated, although there is a need for more specialist, rather large-scale, conferences. There is also a growing interest in management and business-related training and the need to improve the more general induction of new staff into their organization. At a more basic level, biotechnology is also entering secondary school curricula, while a number of undergraduate courses are now under way, although there is little evidence yet of a demand for these specialized courses from industry³.

In the 1990s and beyond, there will have to be increased investment in training and widening of the skills base if biotechnology is to overcome the market, cost and social challenges and so meet its full potential. □

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1. *Biotechnology — Economic and Wider Impacts* (OECD, Paris, 1989).
2. *Training for Employment in Biotechnology* (SERC/IMS, 1989).
3. *Manpower and Training Needs for UK Biotechnology* (Biochemical Society, London, 1989).